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Skin autofluorescence in people with type 1 diabetes and people without diabetes: an eight-decade cross-sectional study with evidence of accelerated aging and associations with complications

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What's new?

- In a cross-sectional study we non-invasively measured skin autofluorescence (SAF) in 269 people with type 1 diabetes and in 114 people of similar age without diabetes across eight decades of age. This is the first SAF study including youths and adults across a large age range.
- Skin autofluorescence increases with age, and faster in diabetes with microvascular complications. SAF in longer-term ex-smokers was comparable to that of non-smokers.
- Skin autofluorescence was higher in women than men in both groups.
- Results support SAF assessment in clinical research and potentially as a screening tool for diabetes complications, particularly for diabetic retinopathy, although longitudinal studies are merited.

Abstract

Aim To measure skin autofluorescence in youths and adults and to assess its relationship with type 1 diabetes, chronic complications and smoking.

Methods In a cross-sectional study (n = 383) skin autofluorescence was measured in 269 people with type 1 diabetes (67 with vascular complications) and 114 people without diabetes, covering eight decades of age. Associations of skin autofluorescence with demographics and traditional risk factors were assessed.

Results Skin autofluorescence increased with age in people with diabetes: for those with complications it increased by a mean ± SE of 0.029 ± 0.003 arbitrary units per year (r = 0.76) and, for those without complications, it increased by 0.028 ± 0.002 arbitrary units (r = 0.77). These increases were higher than for people without diabetes, whose skin autofluorescence increased by 0.022 ± 0.002 arbitrary units (r = 0.78) per year (P = 0.004). Mean ± SE age-adjusted skin autofluorescence was higher in people with diabetes complications vs people without diabetes complications (1.85 ± 0.04 vs 1.66 ± 0.02 arbitrary units) and people without diabetes (1.48 ± 0.03 arbitrary units; all P<0.0001). Age-adjusted skin autofluorescence was higher in current smokers

and recent ex-smokers vs non-smokers and longer-term ex-smokers (1.86±0.06 vs 1.63±0.02 arbitrary units; $P = 0.0005$). Skin autofluorescence area under the receiver-operating characteristic curve was 0.89 (95% CI 0.85–0.94) for retinopathy and 0.56 (95% CI 0.47–0.65) for nephropathy.

Conclusions Skin autofluorescence increases with age, but faster in people with diabetes, particularly in those with complications and in smokers, consistent with accelerated aging. Skin autofluorescence may facilitate complication screening and prediction. Longitudinal studies are merited.

Introduction

Hyperglycaemia promotes the formation of advanced glycation end-products (AGEs) and chronic diabetes complications. AGEs, a family of compounds produced through the Maillard reaction, accumulate in long-lived proteins such as skin collagen, lens and vascular tissue. Specific skin AGEs (e.g. pentosidine) exhibit intrinsic fluorescence and can be measured non-invasively by skin autofluorescence (SAF) or biochemically in collagen from full-thickness skin biopsies. SAF correlates with both fluorescent and non-fluorescent AGEs in skin collagen [1], and has been correlated with mean HbA_{1c} levels measured up to 5 years previously [2,3].

Higher skin collagen AGEs, measured biochemically from skin biopsies in the type 1 diabetes Diabetes Complications and Control Trial/Epidemiology of Diabetes Interventions and Control (DCCT/EDIC) cohort, have been associated with concurrent and future micro- and macrovascular complications, independent of recent HbA_{1c} levels [4–6]. In the DCCT/EDIC cohort SAF (measured non-invasively using a different system) was associated with recently determined retinopathy, cardiac autonomic neuropathy, clinical neuropathy, nephropathy (albuminuria) and coronary artery calcification but, after adjustment for HbA_{1c}, most associations became non-significant, except for sustained urinary albumin excretion rate >30 mg/24 h and coronary artery calcification [7]. Most diabetes SAF studies have been performed in adults [8], and have found higher SAF to be associated with micro- and macrovascular complications. We previously reported a cross-sectional study of SAF in 135 adolescents with diabetes, finding an association of higher SAF levels with early retinopathy (defined as >1 microaneurysm) and cardiac autonomic neuropathy [2]. In adults without diabetes and adults with type 1 diabetes we also demonstrated inverse correlations between SAF and non-invasive small and large artery elasticity [9]. To our knowledge, there are no studies examining SAF across a wide age range, including youths and adults without diabetes and youths and adults with type 1 diabetes, with and without diabetes complications.

Research design and methods

Study participants

The 383 participants included 269 people with type 1 diabetes and 114 people without diabetes. Youths with diabetes duration ≥2 years (*n*=123) were recruited from the Children's Hospital at Westmead (CHW) and adults with diabetes (*n*=146) were recruited from St Vincent's Hospital (SVH) diabetes outpatient clinics during 2001–2014. Adults without diabetes (*n*=81) were recruited from a research volunteer registry. Youth without diabetes (*n*=33) were recruited from non-diabetic non-obese siblings and CHW hospital visitors. Adults without diabetes had no current or history of diabetes, no known cardiovascular or renal disease, and normal fasting glucose and HbA_{1c} levels. Adolescents without diabetes had no known diabetes, renal or cardiovascular disease. Smoking status was assessed according to whether the participant was a current smoker (≥1 cigarette/day), an ex- or never-smoker, and the number of smoking pack-years. Biochemistry assays were performed in respective hospital pathology departments. Estimated GFR was calculated using the Cockroft–Gault formula.

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Chronic complications

Participants were classified as being free from clinically significant vascular complications or having vascular complications if ≥1 microvascular (retinopathy or nephropathy) complication or cardiovascular disease was present. In clinical practice and in our previous research [2,9,10], different definitions of microvascular complications are often used for adults and youths. In adults, retinopathy is defined as clinically significant proliferative retinopathy or retinopathy requiring laser treatment. Retinopathy in youth is often defined as having ≥1 microaneurysm/haemorrhage in either eye, assessed by the Early Treatment of Diabetic Retinopathy Study adaptation of the modified Arlie House classification (level 21, non-proliferative diabetic retinopathy or greater). In the present study, retinopathy was defined as clinically significant proliferative retinopathy or retinopathy requiring laser treatment or intraocular anti-VEGF injections. In adults, diabetic nephropathy was defined by albuminuria >15 μg/min in at least two of three 12- or 24-h urine collections at any time pre-study. Even if albuminuria had been treated and normalized, individuals were still regarded as having nephropathy. In youths, diabetic nephropathy was defined by albuminuria >7.5 μg/min from three overnight timed urine collections. This cut-off is above the 95th percentile of the normal adolescent population and has been shown to be predictive of albuminuria. Given the challenges of timed urine collections, a spot urine albumin/creatinine ratio and eGFR on the study visit day is reported.

Only six adults and no youths had macrovascular complications [cardiovascular disease (defined as acute myocardial infarction, angina or positive Rose questionnaire], transient ischaemic attack, stroke or vascular bypass procedure], all of whom also had microvascular complications.

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Skin autofluorescence

Skin autofluorescence was measured using the Skin AGE Reader (DiagnOptics, Groningen, The Netherlands), as previously [2,9]. Three readings were taken from unscarred skin on the volar surface of each arm, corrected for skin colour, and the mean of six readings [arbitrary units (AU)] used in data analyses. For adults, SAF was assessed after an overnight fast before medication or smoking. Youths had variable fasting status.

Statistics

Data were analysed using XLSTAT package (Addinsoft, Paris, France) and Statistica for Windows (Tibco Software, Tulsa, OK, USA). Data are presented as *n* (%), mean ± SD or median (quartiles), with the exception of age-adjusted SAF which is presented as mean ± SE. *P* values <0.05 were taken to indicate statistical significance. Differences between groups were analysed using Student's *t*-test, and ANOVA, with a Tukey *post hoc* test to retain type 1 error. Correlations were assessed using Pearson or Spearman coefficients. Non-normally distributed variables (triglycerides, eGFR and urinary albumin/creatinine ratio) were analysed using non-parametric tests (Kruskal–Wallis ANOVA). Frequency data in categorical variables were analysed using chi-squared tests. SAF comparisons were performed after controlling for age, sex, HbA_{1c}, smoking and diabetes status (where indicated) by linear models. Multivariable regression with backward selection was used to find independent determinants of SAF for people with diabetes, selected from a model including age, sex, BMI, concurrent HbA_{1c}, mean arterial pressure, pulse pressure, total cholesterol, triglycerides, HDL cholesterol, eGFR, smoking and complication status. Triglycerides and eGFR values were log-transformed. Logistic regression was used to assess associations between SAF and other risk factors and diabetes complications status. Receiver-operating characteristic (ROC) curve analysis was used to assess the discrimination ability of SAF for diabetes complications. Internal validation was used for all models using 10-fold repeated cross-validation.

Ethics

This cross-sectional study was approved by the Human Research Ethics Committees of the CHW and SVH, Melbourne. Written informed consent was obtained from adults. For youths, verbal assent was obtained as well as written consent from their legal guardian.

Results

Participant characteristics

Table 1 describes people without diabetes and people with type 1 diabetes stratified by complication status. Characteristics of youths and adults are shown separately in Tables S1 and S2. Of 67 participants with microvascular complications, 14 had retinopathy alone, 38 had nephropathy alone, and 15 had both. People with diabetes complications had longer diabetes duration, higher HbA_{1c}, higher blood pressure and lower HDL cholesterol levels (all $P < 0.01$) than those without complications.

Increased skin autofluorescence with age

Skin autofluorescence increases linearly with age and at higher rates in diabetes groups both with (slope coefficient of SAF increase per 1 year of age: 0.029 ± 0.003, $r = 0.76$) and without complications (slope coefficient 0.028 ± 0.002, $r = 0.77$) than in people without diabetes (0.022 ± 0.002, $r = 0.78$; Fig. 1). The rates did not differ significantly by diabetes complications status.

We compared the chronological ages for people with diabetes with similar SAF between those with and without complications to people without diabetes (estimates obtained using SAF vs age regression). Similar SAF levels did not occur in people without diabetes until much older chronological ages. For example, SAF in the diabetes with complications group at ages 20, 40 and 60 years were comparable to the SAF of people without diabetes aged ~33.1, 59.6 and 86.1 years, respectively. SAF in the diabetes without complications at ages 20, 40 and 60 years was comparable to that of people without diabetes aged ~25.5, 50.9 and 76.4 years, respectively. SAF in the diabetes with complications group aged 20, 40 and 60 years was similar to that of the diabetes without complications group aged ~26.0, 46.8 and 67.7 years.

Sex and skin autofluorescence

Including all people without diabetes and people with diabetes (mean±SD), SAF was higher in women 1.72 ± 0.57AU than in men: 1.50 ± 0.52 AU ($P<0.001$), and significance remained after controlling for age and smoking status. In people without diabetes, unadjusted SAF in women vs men was 1.63 ± 0.45 AU vs 1.40 ± 0.46 AU ($P = 0.009$), respectively. In people with diabetes unadjusted SAF was 1.76 ± 0.61 AU in women and 1.54 ± 0.54 AU in men ($P=0.003$). Table S3 shows the results of the sex and SAF analyses, controlled for age, smoking and diabetes status.

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Increased skin autofluorescence with diabetes and its vascular complications

The diabetes group had significantly higher SAF than the group without diabetes. Table 1 shows age-adjusted SAF in people without diabetes and people with diabetes, stratified by complication status. SAF differed among people without diabetes, and people with diabetes without and with complications (1.48 ± 0.03 vs 1.66 ± 0.02 vs 1.85 ± 0.04 AU; P<0.0001). Relative to people without diabetes, SAF was significantly higher in the complication-free diabetes group. In the diabetes with complications group, SAF was significantly higher than in people without diabetes and people with complication-free diabetes. Based on 'adult' criteria, only 26 youths had diabetes complications (all renal), vs 40 if 'paediatric' retinopathy criteria were used.

Correlates of skin autofluorescence

In people without diabetes, SAF correlated significantly with higher age, BMI, systolic blood pressure, mean arterial pressure and concurrent HbA_{1c}, total and LDL cholesterol and with lower eGFR. In people with diabetes, SAF correlated significantly with age, diabetes duration, BMI, systolic, diastolic and mean blood pressure, pulse pressure, serum creatinine and lower eGFR (Table 2). In multivariable regression, significant independent determinants of SAF in diabetes were age, sex, mean arterial pressure, HbA_{1c}, HDL cholesterol, eGFR, smoking and complication status (Table S4).

Smoking and skin autofluorescence

Twenty-one adults (including 13 with diabetes) who were current smokers self-reported a median (quartiles) of 50 (15, 100) pack-years. There were 44 ex-smokers (27 with diabetes), with 70% reported having stopped smoking >5 years ago. Ex-smokers reported smoking a median (quartiles) of 68 (15, 275) pack-years. There were no sex differences in proportions of current, ex- and never-smokers. After controlling for age and diabetes status, SAF differed by smoking status. SAF was higher in current smokers vs ex- and never-smokers: 1.98 ± 0.08 AU vs 1.75 ± 0.06 AU and 1.75 ± 0.02 AU, respectively (P = 0.01). SAF differed between ex-smokers who stopped smoking <5 vs >5 years ago (2.03 ± 0.10 AU vs 1.68 ± 0.07 AU; P = 0.003). SAF in those who never smoked was lower than in ex-smokers who recently stopped smoking (<5 years earlier; 1.81 ± 0.03 AU vs 2.03 ± 0.10 AU; P=0.02), but was not different from those who stopped smoking >5 years earlier (1.81 ± 0.03 AU vs 1.68 ± 0.07 AU; P=0.09). There were no significant differences in SAF between smokers and ex-smokers who stopped smoking ≤5 years ago (2.03 ± 0.08 AU vs 2.03 ± 0.10 AU; P=0.99).

Skin autofluorescence and associations with diabetes microvascular complications

In logistic regression, SAF was a significant determinant of retinopathy status, increasing the area under the ROC curve (AUC) when incorporated into other models (Table S5). Using SAF alone as a discriminator of the presence of any retinopathy, nephropathy or macrovascular complication, the AUC was 0.65 [95% CI 0.55, 0.74; *P*=0.002 (Fig. 2a)]. For retinopathy, the AUC was 0.89 (95% CI 0.84, 0.94; *P*<0.0001), reaching a sensitivity of 0.75 (0.69, 0.80) and a specificity of 0.93 (0.76, 0.99) at the SAF cut-off level of 1.82 AU. For nephropathy, the AUC was 0.56 (95% CI 0.47, 0.65; *P*=0.19), with maximum sensitivity and specificity of 0.75 (0.68, 0.80) and 0.40 (0.28, 0.53), respectively, at the SAF cut-off level of 1.96 AU (Fig. 2a). If adult and paediatric age-specific retinopathy criteria were used, the significant difference remained, and the AUC for overall complication discrimination was 0.61 (95% CI 0.53, 0.68; *P*=0.004), and for retinopathy it was 0.69 (95% CI 0.61, 0.77; *P*<0.0001), with a maximum sensitivity 0.65 (0.58, 0.71) and specificity of 0.68 (0.54, 0.79) at the SAF cut-off level of 1.65 AU (Fig. 2b).

Discussion

In this cross-sectional study including people without diabetes and people with type 1 diabetes aged 9–73 years, SAF measured non-invasively increased with chronological age and at a faster rate in those with diabetes. Age-adjusted SAF was higher in people with diabetes either with or without complications than in people without diabetes, and was higher in people with diabetes and complications than in complication-free people with diabetes. Smokers had significantly higher SAF levels than non-smokers, but longer-term (>5 years) ex-smokers had similar SAF levels to non-smokers.

Diabetes is described as a condition of accelerated aging [11]. Our findings of higher SAF with older age in people without diabetes and in people with diabetes, using a non-invasive device that could be used in clinical practice, replicate and extend into the paediatric age group prior research by Verzijl *et al.* [12]. Verzijl *et al.* demonstrated that skin collagen fluorescence and AGEs in full-thickness skin biopsies increased in 27 people without diabetes aged 19–91 years. Our participants' ages ranged from 9 to 67 years for those without diabetes and 10 to 73 years for those with diabetes. In similar skin biopsy studies by Dyer *et al.* [13] collagen total fluorescence, resistance to acid hydrolysis, and specific AGEs were determined biochemically by gas chromatography/mass spectroscopy and high-pressure liquid chromatography [13]. While highly sensitive and specific, this 'gold standard' analysis technique requires a skin biopsy with local anaesthetic and sutures, as well as costly instrumentation, time, and skilled analysts. In a validation study by the Baynes Laboratory and the SAF system developer, there was very good correlation between non-invasive SAF and both fluorescent and non-fluorescent AGEs in biopsied human skin collagen [14].

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We used a non-invasive SAF system, which in previous clinical research, usually in adults, has demonstrated increased SAF levels in people with vs people without type 1 or type 2 diabetes and chronic complications, including foot ulcers in people with type 2 diabetes with metabolic syndrome, insulin resistance in type 1 diabetes, and in people with an abdominal aortic aneurysm or aortic stiffening [15–17]. Importantly, SAF can predict chronic renal disease, mortality in people with end-stage renal disease, amputations in peripheral vascular disease, peripheral neuropathy, cardiorenal outcomes and cardiovascular mortality in people with diabetes [18,19]. Using the same SAF system, we previously demonstrated positive linear correlations between skin and ocular autofluorescence and negative linear correlations between SAF and small artery elasticity measured non-invasively in adults with diabetes and people without diabetes [9].

We noted higher SAF in women than men; however, significance was not evident when adults and youths without diabetes were analysed separately. Several groups have also demonstrated sex differences in SAF, with higher levels in women than men [20,21]. Mook-Kanamori *et al.* [21] reported higher SAF in women with type 2 diabetes and women without diabetes [21]. Higher SAF in women was also reported in a Saudi population, with no mention of diabetes status [20]. In most papers, sex differences in SAF are not reported [22,23]. To our knowledge we are the first to report SAF sex differences in diabetes. These sex differences were observed only in people with diabetes and not in those without diabetes. Potential contributing factors are that preteen girls tend to have worse glycaemia than pre-teen boys [24], and there are years of metabolic memory for glycaemia, which probably impacts SAF as skin collagen turnover is ~15 years [12]. There is also potential for lifestyle differences, such as sun exposure, smoking and coffee consumption, to increase SAF, but we do not have relevant data.

There is a substantial literature demonstrating higher SAF in people with diabetes complications [19,25–27], reviewed by Fokkens and Smit [18] and by Bos *et al*. [16]. All studies showed an association of SAF with ≥1 chronic diabetes complication. Our results are confirmatory. Our findings also support the idea that SAF may be used as a screening tool for retinopathy, perhaps in disadvantaged regions where retinopathy screening is not widely available, reaching a specificity of >95%. We previously reported that higher SAF in adolescents with diabetes is associated with (early) retinopathy and cardiac autonomic dysfunction [2]. Also supporting use of SAF as a screening tool is the finding from a recent LifeLines Cohort study that SAF improved the Finnish Diabetes Risk Score (for type 2 diabetes detection) [28].

Smoking is a source of exogenous AGEs. We identified (in a combined group of people without and people with diabetes) higher SAF in smokers than non-smokers, and divergence in SAF amongst ex-smokers based on how long ago they had stopped smoking. We chose a threshold based on the skin collagen half-life of ~15 years [12], which translates to turnover of ~20% of skin collagen over 5 years and on smoking cessation duration, where 5 years of non-smoking reduces total mortality hazard ratio by 35% and cardiovascular disease mortality by 12%. We noted that SAF was 18% lower in those with a moderate time since smoking cessation than in smokers. Another cross-sectional study shows that in people with ~15 years of smoking cessation, SAF is similar to that in those who have never smoked [29]. Longitudinal studies are desirable but will take many years.

Strengths of the present study include: use of the same SAF system by only four operators in two sites, with no operator-related differences (data not shown); our assessment of the lack of effect of prandial status on SAF; the wide age range, covering paediatric and adult groups; the study size; inclusion of smoking status, albeit self-reported; and assessment of diabetes complications by both adult criteria and by age-differential criteria. Limitations include the cross-sectional design and lack of blood and urine testing of youths without diabetes. We did not assess neuropathy status due to its less objective clinical criteria than for nephropathy and retinopathy. Coexistence of neuropathy with renal or retinal complications would not change our results, but if some participants otherwise classified as complication-free had neuropathy alone, it would bias against us finding between-group differences.

In conclusion, using a well-accepted, well-validated non-invasive measure, we analysed SAF in relationship to diabetes and its chronic complications in youths and adults spanning eight decades of age. The rate of SAF increase was greater in people with diabetes vs people without diabetes. Age, sex, mean arterial pressure, HbA_{1c}, HDL cholesterol, eGFR, smoking and diabetes complication status were significant determinants of SAF in diabetes. Evidence is emerging to consider SAF use in clinical research and perhaps, if shown useful, in clinical practice. Further longitudinal and intervention studies would be of particular interest, such as those related to improved glycaemia and with insulin pump use [30].

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Competing interests
None declared.

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Table 1 Clinical and biochemical characteristics of people without diabetes and people with type 1 diabetes, stratified by complications status

	No diabetes	Diabetes without vascular complications	Diabetes with vascular complications	*P***
N	114	202	67	-
Ethnicity: white	111 (97)	190 (94)	64 (96)	**0.84**
Age, years	31±16	26±14	31±18	**0.01**
Sex: male	54 (47)	96 (48)	35 (52)	0.50
Diabetes duration, years	-	12±9	20±14	**<0.0001**
HbA_{1c} , mmol/mol	33±3††	65±15	70±17	**0.03**
HbA_{1c}, %	5.1±0.3†	8.1±1.4	8.6±1.6	**0.03**
BMI, kg/m^2	25.2±4.5	24.4±4.2	25.6±5.7	**0.06**
Mean arterial blood pressure , mmHg	89±14††	83±11	89±14	**0.0008**
Systolic blood pressure, mmHg	121±17†	116±14	123±20	**0.004**
Diastolic blood pressure, mmHg	69±11††	64±9	69±11	**0.003**
Pulse pressure , mmHg	53±11††	52±11	54±14	0.16
Total cholesterol, mmol/l	5.1±1.0††	4.5±1.0	4.3±1.0	0.34
Triglycerides, mmol/l	1.1 (0.8, 1.4) ††	0.9 (0.7, 1.3)	1.0 (0.8, 1.4)	0.11
HDL- cholesterol, mmol/l	1.5±0.4††	1.5±0.3	1.3±0.4	**0.02**
LDL- cholesterol, mmol/l	3.0±0.9††	2.5±0.8	2.4±0.9	0.71
eGFR, ml/min/1.73m^2	106 (93, 124)††	122 (103, 143)	123 (100, 136)	0.59
Urine albumin/creatinine ratio, mg/mmol	0.43 (0.30, 0.80) ††	0.54 (0.38, 0.89)	1.51 (0.70, 3.33)	**<0.0001**
Serum creatinine, µmol/l	75±15††	65±15	78±47	**0.002**
Current smokers/<5 years ex-smokers /≥5 years ex-smokers	8/5/11 (7/4/10)	14/6/11 (7/3/5)	0/4/9 (0/6/13)**	**0.04**
SAF‡ (, AU)***	1.48±0.03	1.66±0.02	1.85±0.04	**0.0001**

SAF, skin autofluorescence.

**P*<0.05 vs people without type 1 diabetes. ***P* for comparison people with type 1 diabetes without vascular complications and people with type 1 diabetes with vascular complications. †Results available only from adults without type 1 diabetes. ‡Age-adjusted.

Data are presented as: *n* (%); mean ± SD (mean ± SE for age-adjusted SAF) and median (quartiles).

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Table 2 Correlations (Pearson) of skin autofluorescence in people without diabetes and people with type 1 diabetes, stratified by complications status

	No diabetes, *n*=114		Diabetes without vascular complications, *n*=202		Diabetes with vascular complications, *n*=67	
	r	*P*	*r*	*P*	*r*	*P*
Age, years	0.78	**<0.0001**	0.77	**<0.0001**	0.76	**<0.0001**
Diabetes duration, years	-		0.60	**<0.0001**	0.72	**<0.0001**
HbA_{1c}, mmol/mol	0.32	**0.009**	-0.16	**0.03**	-0.07	0.57
HbA_{1c}, %	0.32	**0.009**	-0.16	**0.03**	-0.07	0.57
BMI, kg/m^2	0.22	**0.03**	0.30	**<0.0001**	0.47	**<0.0001**
Mean arterial blood pressure, mmHg	0.30	**0.01**	0.46	**<0.0001**	0.58	**<0.0001**
Systolic blood pressure, mmHg	0.29	**0.01**	0.30	**<0.0001**	0.59	**<0.0001**
Diastolic blood pressure, mmHg	0.22	0.06	0.38	**<0.0001**	0.38	**0.002**
Pulse pressure, mmHg	0.15	0.20	0.09	0.22	0.53	**<0.0001**
Total cholesterol, mmol/l	0.36	**0.002**	0.20	**0.005**	-0.04	0.74
Triglycerides, mmol/l	0.21	0.08	-0.05	0.50	-0.16	0.19
HDL cholesterol, mmol/l	0.02	0.85	0.14	**0.04**	0.003	0.99
LDL cholesterol, mmol/l	0.32	**0.006**	0.18	**0.01**	0.04	0.77
eGFR, ml/min/1.73m^2	–0.28	**0.02**	–0.16	**0.02**	–0.25	0.05
Urine albumin/creatinine ratio, mg/mmol	0.13	0.29	-0.003	0.96	0.01	0.95
Serum creatinine, µmol/l	–0.07	0.59	0.27	**0.0002**	0.33	**0.01**

For triglycerides, eGFR and urinary albumin/creatinine ratio, Spearman correlations results are shown.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Clinical and biochemical characteristics of adolescents (under 18 years) without diabetes and adolescents with type 1 diabetes, stratified by complications status.

Table S2 Clinical and biochemical characteristics of adults (≥ 18 years) without diabetes and with type 1 diabetes, stratified by complications status.

Table S3 Skin autofluorescence comparison in men and women after adjustment for age and smoking status.

Table S4 Determinants of SAF in type 1 diabetes *n*=269 (multivariable regression analysis). Adjusted* R^2=0.68, P<0.0001. Variables used to establish the model: age, sex, BMI, concurrent HbA_{1c}, mean arterial pressure, pulse pressure, total cholesterol, triglycerides, HDL cholesterol, eGFR, smoking status and complication status.

Table S5 Logistic regression models for estimation of an association between skin autofluorescence (with addition of age, sex and HbA_{1c}) and diabetic retinopathy defined using diabetic retinopathy diagnostic criteria for adults. N = 269 (including 29 with diabetic retinopathy). Odds ratio for skin autofluorescence are shown per 0.1 AU change. AUC cross-validation calculated from 10-fold AUC cross-validation repeated 1000 times.

FIGURE 1 Correlation between age and skin autofluorescence in people without diabetes and people with type 1 diabetes with and without vascular complications. Slope coefficients ± SE: 0.022±0.002, 0.028±0.002 and 0.029±0.003, respectively (type 1 diabetes without vascular complications vs. type 1 diabetes with vascular complications; P =0.74). AU, arbitrary units.

FIGURE 2 Receiver-operating characteristic curves for diabetes vascular complication status, nephropathy and retinopathy determination using skin autofluorescence. (a) Adult criteria used for diagnosis of diabetic retinopathy. (b) Age-specific criteria used for peer review diagnosis of diabetic retinopathy. AUC, area under the receiver-operating characteristic curve.

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Table 1.

Clinical and biochemical characteristics of people without diabetes and people with type 1 diabetes, stratified by complications status.

	No diabetes	Diabetes without vascular complications	Diabetes with vascular complications	p**
N	114	202	67	-
Ethnicity (Caucasian)	111 (97)	190 (94)	64 (96)	**0.84**
Age (years)	31±16	26±14	31±18	**0.01**
Sex (male)	54 (47)	96 (48)	35 (52)	0.50
Diabetes duration (years)	-	12±9	20±14	**<0.0001**
HbA1c (%)	5.1±0.3†	8.1±1.4	8.6±1.6	**0.03**
HbA1c (mmol/mol)	33±3†	65±15	70±17	**0.03**
BMI (kg/m^2)	25.2±4.5	24.4±4.2	25.6±5.7	**0.06**
Mean arterial blood pressure (mmHg)	89±14†	83±11	89±14	**0.0008**
Systolic blood pressure (mmHg)	121±17†	116±14	123±20	**0.004**
Diastolic blood pressure (mmHg)	69±11†	64±9	69±11	**0.003**
Pulse pressure (mmHg)	53±11†	52±11	54±14	0.16
Total cholesterol (mmol/L)	5.1±1.0†	4.5±1.0	4.3±1.0	0.34
Triglycerides (mmol/L)	1.1 (0.8, 1.4) †	0.9 (0.7, 1.3)	1.0 (0.8, 1.4)	0.11
HDL-cholesterol (mmol/L)	1.5±0.4†	1.5±0.3	1.3±0.4	**0.02**
LDL-cholesterol (mmol/L)	3.0±0.9†	2.5±0.8	2.4±0.9	0.71
eGFR (ml/min/1.73m^2)	106 (93, 124) †	122 (103, 143)	123 (100, 136)	0.59
Urine albumin / creatinine ratio (mg/mmol)	0.43 (0.30, 0.80) †	0.54 (0.38, 0.89)	1.51 (0.70, 3.33)	**<0.0001**
Serum creatinine (μmol/L)	75±15†	65±15	78±47	**0.002**

Current smokers/<5yrs ex-smokers /≥5yrs ex-smokers	8/5/11 (7/4/10)	14/6/11 (7/3/5)	0/4/9 (0/6/13)**	**0.04**
Skin autofluorescence (AU)***	1.48±0.03	1.66±0.02	1.85±0.04	**0.0001**

† - results available only from adults without type 1 diabetes

* - p<0.05 vs. people without type 1 diabetes

** - p for comparison people with type 1 diabetes without vascular complications and people with type 1 diabetes with vascular complications

*** - age-adjusted, mean±SE

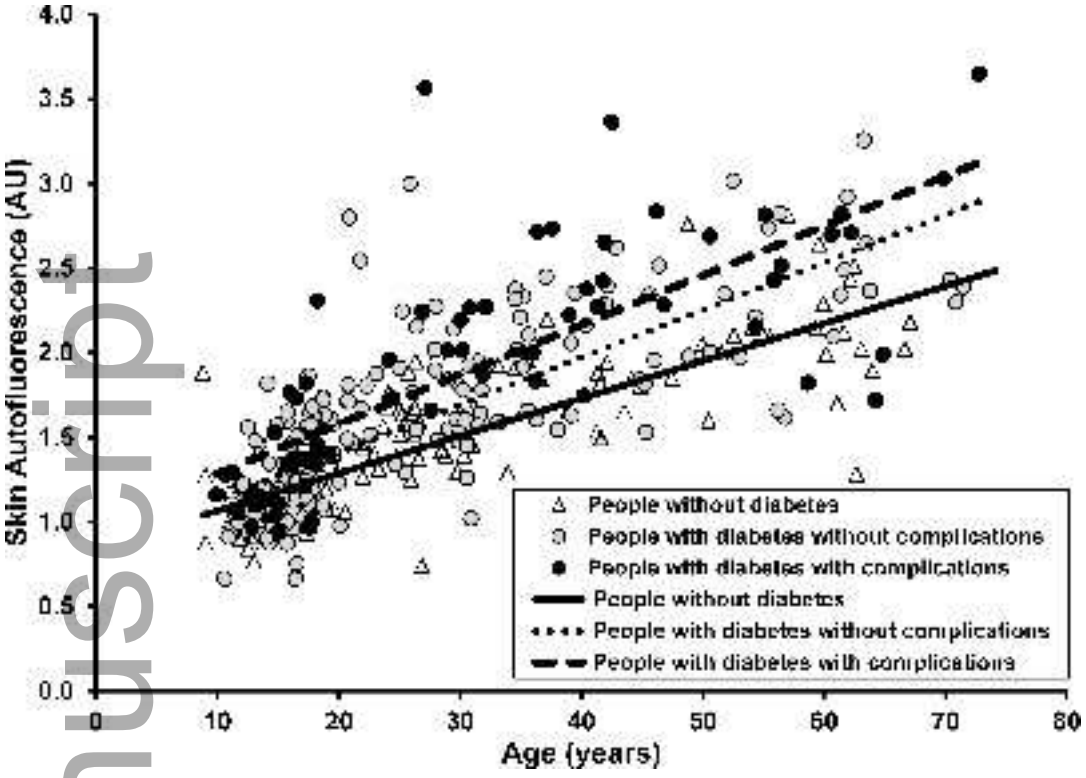
Data are presented as n (%), mean±SD, median (quartiles); for age adjusted skin autofluorescence as mean±SE.

Table 2.

Correlations (Pearson) of skin autofluorescence in people without diabetes and people with type 1 diabetes, stratified by complications status.

For triglycerides, eGFR and urinary albumin/creatinine ratio, Spearman correlations results are shown.

	No diabetes n=114		Diabetes without vascular complications n=202		Diabetes with vascular complications n=67	
	r	p	r	p	r	p
Age (years)	0.78	**<0.0001**	0.77	**<0.0001**	0.76	**<0.0001**
Diabetes duration (years)	-		0.60	**<0.0001**	0.72	**<0.0001**
HbA1c (%)	0.32	**0.009**	-0.16	**0.03**	-0.07	0.57
HbA1c (mmol/mol)	0.32	**0.009**	-0.16	**0.03**	-0.07	0.57
BMI (kg/m^2)	0.22	**0.03**	0.30	**<0.0001**	0.47	**<0.0001**
Mean arterial blood pressure (mmHg)	0.30	**0.01**	0.46	**<0.0001**	0.58	**<0.0001**
Systolic blood pressure (mmHg)	0.29	**0.01**	0.30	**<0.0001**	0.59	**<0.0001**
Diastolic blood pressure (mmHg)	0.22	0.06	0.38	**<0.0001**	0.38	**0.002**
Pulse pressure (mmHg)	0.15	0.20	0.09	0.22	0.53	**<0.0001**
Total cholesterol (mmol/L)	0.36	**0.002**	0.20	**0.005**	-0.04	0.74
Triglycerides (mmol/L)	0.21	0.08	-0.05	0.50	-0.16	0.19
HDL-cholesterol (mmol/L)	0.02	0.85	0.14	**0.04**	0.003	0.99
LDL-cholesterol (mmol/L)	0.32	**0.006**	0.18	**0.01**	0.04	0.77
eGFR ($ml/min/1.73m^2$)	-0.28	**0.02**	-0.16	**0.02**	-0.25	0.05
Urine albumin / creatinine ratio (mg/mmol)	0.13	0.29	-0.003	0.96	0.01	0.95
Serum creatinine (μmol/L)	-0.07	0.59	0.27	**0.0002**	0.33	**0.01**



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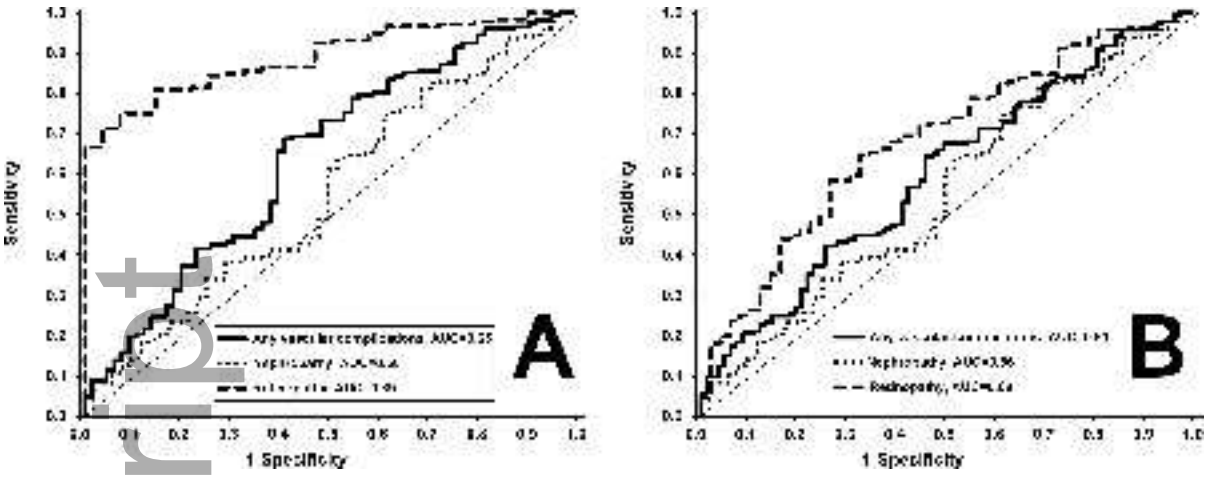

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