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ORIGINAL ARTICLE

Caffeine Consumption Contributes to Skin Intrinsic Fluorescence in Type 1 Diabetes

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Abstract

Background: A variant (rs1495741) in the gene for the *N*-acetyltransferase 2 (NAT2) protein is associated with skin intrinsic fluorescence (SIF), a noninvasive measure of advanced glycation end products and other fluorophores in the skin. Because NAT2 is involved in caffeine metabolism, we aimed to determine whether caffeine consumption is associated with SIF and whether rs1495741 is associated with SIF independently of caffeine.
Materials and Methods: SIF was measured in 1,181 participants with type 1 diabetes from the Epidemiology of Diabetes Interventions and Complications study. Two measures of SIF were used: SIF1, using a 375-nm excitation light-emitting diode (LED), and SIF14 (456-nm LED). Food frequency questionnaires were used to estimate mean caffeine intake. To establish replication, we examined a second type 1 diabetes cohort.
Results: Higher caffeine intake was significantly associated with higher $SIF1_{LED\ 375\,nm[0.6,\,0.2]}$ ($P=2\times10^{-32}$) and $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$ ($P=7\times10^{-31}$) and accounted for 4% of the variance in each after adjusting for covariates. When analyzed together, caffeine intake and rs1495741 both remained highly significantly associated with $SIF1_{LED\ 375\,nm[0.6,\,0.2]}$ and $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$. Mean caffeinated coffee intake was also positively associated with $SIF1_{LED\ 375\,nm[0.6,\,0.2]}$ ($P=9\times10^{-12}$) and $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$ ($P=4\times10^{-12}$), but no association was observed for decaffeinated coffee intake. Finally, caffeine was also positively associated with $SIF1_{LED\ 375\,nm[0.6,\,0.2]}$ and $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$ ($P<0.0001$) in the replication cohort.
Conclusions: Caffeine contributes to SIF. The effect of rs1495741 on SIF appears to be partially independent of caffeine consumption. Because SIF and coffee intake are each associated with cardiovascular disease, our findings suggest that accounting for coffee and/or caffeine intake may improve risk prediction models for SIF and cardiovascular disease in individuals with diabetes.

Introduction

Measurement of skin intrinsic fluorescence (SIF) represents a novel noninvasive biomarker of advanced glycation end products (AGEs) and other fluorophores in the skin.[1,2] Skin fluorescence has been shown to be positively associated with subclinical atherosclerosis and/or cardiovascular disease mortality in the general population,[3] in type 1 and type 2 diabetes,[4–6] and in people with renal failure.[7]

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The DCCT and the EDIC are registered at ClinicalTrials.gov with clinical trial registration numbers NCT00360815 and NCT00360893, respectively.
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Several factors contribute to interindividual variability in skin fluorescence, including age, glycated hemoglobin (HbA1c), smoking and nephropathy.[7,8] Recently, rs1495741 in the gene for the *N*-acetyltransferase 2 (NAT2) protein, which tags arylamine *N*-acetyltransferase enzyme activity status,[9] was identified as a major locus influencing skin fluorescence across four cohorts consisting of subjects with and without diabetes, explaining up to 15% of the variance.[10] NAT2 is a phase II drug-metabolizing enzyme that metabolizes aromatic amine and hydrazine drugs, heterocyclic amines,[11] and caffeine following initial detoxification by CYP1A2.[12] *NAT2* has been previously associated with serum 1-methylxanthine:4-acetamidobutanoate ($P=1.7\times10^{-40}$) in a genome-wide association study (GWAS).[13] This metabolite ratio likely reflects the role of NAT2 in the metabolism of caffeine because caffeine is metabolized to 1-methylxanthine, which is used to determine the NAT2 acetylator status.[12] Because caffeine exhibits fluorescent properties[14] and NAT2 metabolizes caffeine metabolites, it is plausible that the *NAT2* association with SIF is due to fluorescence of caffeine in the skin. We therefore tested whether caffeine consumption is associated with SIF and whether the association of rs1495741 with SIF is independent of caffeine intake. We separately used two cohorts of subjects with type 1 diabetes to determine the reproducibility of our observations.

Materials and Methods

Subjects

Subjects included 1,441 patients with type 1 diabetes between 13 and 39 years of age at baseline, who were recruited from 1983 to 1989 to participate in the Diabetes Control and Complications Trial (DCCT).[15] Participants were randomized to intensive ($n=711$) or conventional ($n=730$) therapy and were followed up for a mean of 6.5 years until the study ended in 1993. In 1994, 1,375 subjects (96% of the surviving cohort) were enrolled in the annual observational follow-up study, Epidemiology of Diabetes Interventions and Complications (EDIC).[16] In EDIC year 16 or 17, subjects were invited to participate in the SCOUT substudy to measure SIF, and 92% ($n=1,185$) of the active EDIC subjects participated.[8]

Measurement of SIF

The SCOUT DS® SF spectrometer (VeraLight, Inc., Albuquerque, NM) was used to measure skin tone (pigmentation) and SIF from the underside of the left forearm. SIF is a measure of skin fluorescence that mathematically corrects for factors such as skin pigmentation, which may affect light absorption and scattering.[4,8,17] For all of our analyses we used $SIF1_{LED\ 375\,nm[0.6,\,0.2]}$ and $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$, which were examined previously in genetic association studies of SIF in DCCT/EDIC[10] and represent the lowest (SIF1 = 375 nm) and highest (SIF14 = 456 nm) light-emitting diode (LED) excitation wavelengths examined in the DCCT/EDIC study. Furthermore, $SIF1_{LED\ 375\,nm[0.6,\,0.2]}$ as measured by the SCOUT DS SF spectrometer is similar to the excitation level (approximately 350–370 nm) commonly used by the AGE Reader (DiagnOptics, Groningen, The Netherlands), another device that measures skin fluorescence.[3,6,7] This level of excitation captures fluorescence of AGEs and has been previously shown to have the strongest association with type 1 diabetes complications in the Pittsburgh Epidemiology of Diabetes Complications (EDC) study.[1,2,5,18]

$SIF1_{LED\ 375\,nm[0.6,\,0.2]}$ was detected over an emission range of 435–655 nm and corrected for light scattering with the reflectance adjusted by the dimensionless excitation and emission exponents, $k_x=0.6$ and $k_m=0.2$, respectively. $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$ was detected over an emission range of 491–655 nm, with reflectance adjusted by $k_x=0.4$ and $k_m=0.8$ emission exponents. The first of two SIF measurements, reported in arbitrary units, was used for analyses.

Measurement of caffeine intake

During the DCCT (1983–1993), diet was assessed using a modified Burke-type diet history[19] at the baseline, 2-year, 5-year, and study end points. Participants were asked by dietitians to describe the meals and snacks they usually had to eat or drink on weekdays and on weekends. Seasonal variations in food intake and foods eaten away from home were also recorded. A staff of trained coders at the Central Nutrition Coding Unit (University of Minnesota, St. Paul, MN) coded the diet histories in a standardized manner, and nutrient intakes were calculated using version 13 (1986) of the Nutrition Coordinating Center nutrient database. The intercoder reliability and the reproducibility of the diet history were assessed at Year 2 of the DCCT.[19]

During EDIC years 1–12 (1994–2006), a 126-item self-administered Harvard food frequency questionnaire (FFQ) was used to assess diet intake biennially.[20] Participants were asked how often they consumed a specified serving size of each food item over the past year. The nine response categories ranged from never or less than once per month to six or more times daily. Nutrient intakes from the FFQ were then calculated to reflect daily intake using the corresponding food item and respective portion size primarily from the U.S. Department of Agriculture nutrient database. Frequency response items were available for caffeinated beverages (coffee, tea, cola, and low-calorie cola) and decaffeinated coffee during EDIC Years 13–15. The validity of the Harvard FFQ was assessed previously in a subset of participants from the Nurses' Health Study by comparing the FFQ with 1-week diet records measured approximately every 3 months during the preceding year. Correlation coefficients for coffee and tea consumption were 0.75 and 0.90, respectively.[21]

Genotyping

The rs1495741 (*NAT2*) genotype was available from the Illumina (San Diego, CA) human 1M beadchip assay, which was used for genome-wide genotyping in the DCCT/EDIC.[10] After exclusion of subjects determined to be admixed using population genetic approaches, 1,081 subjects had both rs1495741 genotype data and SIF measurements available.[10]

Statistical analysis

Caffeine intake measured using the FFQ during the EDIC study was used as our primary measure of caffeine exposure. Over 60% of subjects had four or five measures of caffeine intake from FFQs completed during the EDIC study (range, one to seven), and approximately 50% had three measures of caffeine intake from diet histories completed during the

DCCT (range, one to six). Mean caffeine intake was calculated separately for each subject during the DCCT and the EDIC study. To approximate a normal distribution, mean caffeine intake was square root transformed when used as the outcome variable (Supplementary Figs S1 and S2; Supplementary Data are available online at www.liebertonline.com/dia), whereas $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ were $\log_e$ transformed.[10]

rs1495741 was modeled assuming an additive model. Linear regression was used to examine the relationship of rs1495741 with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ in both unadjusted (M1) and adjusted (M2) models. M2 included age, sex, skin tone, EDIC clinic latitude, smoking status, any estimated glomerular filtration rate $<60\ mL/min/1.73\ m^2$, DCCT eligibility HbA1c, mean DCCT HbA1c, and mean EDIC HbA1c[8] (Supplementary Table S1). The same models were also used to examine the association of mean caffeine intake during the EDIC study and/or the DCCT alone or in combination with SIF. To exclude the possibility of confounding due to total reported daily caloric intake, it was included as a covariate in secondary models after excluding under- and over-reporters, defined as consuming <800 kcal/day and >6,000 kcal/day, respectively. We also additionally adjusted M2 for DCCT treatment group and separately for DCCT prevention cohorts in secondary analyses. To examine whether a nonlinear relationship exists between caffeine intake and SIF, $caffeine^2$ was added to M1 and M2. Linear regression was used to test whether rs1495741 was associated with caffeine intake. To determine whether the association of rs1495741 with SIF differed by smoking status, we tested for interaction between rs1495741 and smoking status (never smokers vs. ever and current smokers).

Unadjusted Spearman correlation, and multiple linear regression, adjusting for M2 covariates, were used to determine the association of mean consumption of each of the caffeinated beverages and decaffeinated coffee frequency with SIF. A value of $P<0.05$ was used to establish statistical significance.

Replication study

Subjects comprised 210 participants (48% male) from the Pittsburgh EDC study of childhood-onset diabetes[22] who had caffeine intake and SIF measured. Diet was assessed at the baseline (1986–1988), 2-year, and 10-year exams using the same Harvard FFQ used in the EDIC study. Mean caffeine intake was calculated for each person and used for analyses.

SIF was initially measured in a pilot group of participants living within 25 miles of the study clinic in 2007–2009 ($n=107$) and subsequently in a substudy of participants undergoing brain imaging in 2010 ($n=65$) and at the 25-year examination in 2011 ($n=38$). By June 30, 2013, a total of 210 subjects had SIF measured and were included in the SIF–caffeine analyses. For participants with two SIF measurements available, only the first measurement was used for analyses. On average, SIF was assessed 17.9 ± 3.0 (SD) years (range, 8.7–26.5 years) after diet was assessed, using a similar protocol as in the DCCT/EDIC study.[5]

DNA was collected during the 2-year exam, and rs1495741 was genotyped using fluorescence polarization with a 5′-ctatcttccagaaagtaaatgtg-3′ forward primer, 5′-tggga aactatcatttaaaacag-3′ reverse primer, and 5-gaagctactgtgaat gccca-3′ fluorescence polarization reverse primer and detected on 2% agarose gel. Polymerase chain reaction conditions were 95°C for 5 min, 35 cycles of (95°C for 30 s, 56°C for 30 s, and 72°C for 30 s), and finally 72°C for 5 min and 10°C hold. For fluorescence polarization an annealing temperature of 60°C using the C/T dye mix was used.

Multiple linear regression adjusting for age, sex, smoking, and estimated glomerular filtration rate $<60\ mL/min/1.73\ m^2$ was used to test for association between rs1495741 with SIF. Spearman correlations were used to examine the relationship for mean caffeine intake with SIF. Multiple linear regression was used to test the joint effects of rs1495741 and mean caffeine intake on SIF including age, sex, smoking, and estimated glomerular filtration rate $<60\ mL/min/1.73\ m^2$ as additional covariates.

Results

Table 1 shows subject characteristics of the DCCT/EDIC participants, separately by the former DCCT treatment group. On average, daily caffeine intake during the EDIC study (Supplementary Fig. S1) was 74 mg/day lower than during the DCCT (Supplementary Fig. S2), but caffeine intake was correlated across DCCT and EDIC time periods ($r=0.74$, $P<0.0001$).

Mean caffeine intake during the EDIC study was positively associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ in unadjusted analyses and accounted for approximately 11% of the variance in each, respectively (Table 2 and Supplementary Fig. S3).

After adjusting for covariates, caffeine was still significantly associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$, but the effect was attenuated, and the variance accounted for by caffeine intake during the EDIC study was 3.8% for $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ and 4.2% for $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ (Table 2).

Because age, smoking status, and mean EDIC HbA1c were all positively associated with both $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ (Supplementary Table S1) and mean EDIC caffeine intake (Supplementary Table S2), we examined whether the attenuation of the caffeine effect on SIF in M2 (Table 2) was explained by adjusting for these variables. Adjusting for age and smoking status largely accounted for the attenuation of the caffeine effect on $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ ($\beta\pm$SE, 0.00031 ± 0.000025; $P=1.9\times10^{-33}$, without adjustments for age and smoking status). Similarly, the attenuation of the caffeine effect on $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ was due to adjustments for age and smoking status as well as EDIC HbA1c ($\beta\pm$SE, 0.00035 ± 0.00003; $P=1.6\times10^{-30}$, without adjustments for age, smoking status, and HbA1c).

Additionally adjusting M2 for mean total caloric intake did not materially alter the association of mean EDIC caffeine intake with either $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ ($P=4.1\times10^{-17}$) or $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ ($P=3.9\times10^{-16}$). Similarly, adjusting for DCCT treatment group or DCCT primary versus secondary prevention cohort did not materially alter the results (data not shown).

We also tested whether the association of caffeine intake with SIF is nonlinear and observed some evidence supporting a quadratic relationship for $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ ($\beta_{linear}\pm$SE, 0.0002 ± 0.00003 [$P=5.8\times10^{-16}$]; $\beta_{quadratic}\pm$SE, $-1.8\times$

Table 1. Characteristics of the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications Participants with Skin Instrinsic Fluorescence (SIF) Measures, Separately by Former DCCT Randomized Treatment Group at the Time SIF Was Measured

	Former INT (n = *612*)	*Former CON* (n = *573*)
Demographic characteristics		
Male sex	317 (52%)	308 (54%)
Age (years)	52 ± 7	51 ± 7
Diabetes duration (years)	30.0 ± 4.9	29.5 ± 4.9
Primary cohort assignment[a]	298 (49%)	294 (51%)
Skin tone (arbitrary units)	260 ± 47	256 ± 49
Clinic latitude (>37°N)[b]	444 (73%)	427 (75%)
Smoking status[c]		
Never	372 (61%)	352 (61%)
Former	154 (25%)	149 (26%)
Current	86 (14%)	72 (13%)
Any eGFR <60 mL/min/1.73 m^2 to date (yes)[d]	40 (7%)	44 (8%)
Glycemic exposure		
DCCT eligibility HbA1c (%) (mmol/mol)	9.1 ± 1.6 (76 ± 17)	8.9 ± 1.6 (74 ± 17)
DCCT mean HbA1c (%) (mmol/mol)	7.2 ± 0.8 (55 ± 9)	9.0 ± 1.3 (75 ± 14)
EDIC mean HbA1c (%) (mmol/mol)	8.0 ± 1.1 (63 ± 12)	8.0 ± 1.0 (63 ± 11)
Time-weighted mean HbA1c (%) (mmol/mol)[e]	8.0 ± 0.9 (64 ± 10)	8.4 ± 0.9 (68 ± 10)
Mean caffeine intake during DCCT (mg/day)	369 ± 329	369 ± 342
Mean caffeine intake during EDIC (mg/d)[f]	301 ± 218	288 ± 209
Time between caffeine intake assessment and SIF1 (years)[g]	11.1 ± 1.1	11.0 ± 1.0
rs1495741 genotype (AA/AG/GG)[h]	342/189/24	307/191/28
$SIF1_{LED\ 375nm,\ kx=0.6,\ km=0.2}$ (arbitrary units)[i]	3.1 ± 0.2	3.1 ± 0.21
$SIF14_{LED\ 456nm,\ kx=0.4,\ km=0.8}$ (arbitrary units)[i]	0.37 ± 0.23	0.36 ± 0.23

Data are *n* (%) or mean ± SD values as indicated (*n* = 1,185).

[a]Two cohorts were recruited at DCCT baseline: a primary cohort (*n* = 726) of subjects with no retinopathy and a urinary albumin excretion rate of <40 mg/24 h at baseline and a secondary cohort (*n* = 715) of subjects exhibiting mild to moderate nonproliferative retinopathy and urinary albumin excretion rate of ≤200 mg/24 h at baseline.

[b]Clinic latitude was categorized as a binary variable with clinics located above 37°N latitude designated as northern clinics (*n* = 21) and those below assigned as southern clinics (*n* = 7).

[c]Smoking status was defined as "never smoker" (≤100 cigarettes in a subject's lifetime), "former smoker" (quit ≥1 year ago), or "current smoker" (currently smoking or smoking within the last year).

[d]Estimated glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease–Epidemiology Collaboration equation.

[e]Time-weighted mean glycated hemoglobin (HbA1c) is calculated by summing (DCCT eligibility HbA1c × duration of diabetes at DCCT baseline), (DCCT mean HbA1c × years of follow-up in DCCT), and (Epidemiology of Diabetes Interventions and Complications [EDIC] mean HbA1c × years of follow-up in EDIC) and dividing by total duration of diabetes.

[f]Two subjects in the intensive treatment (INT) group and two in the conventional treatment (CON) group did not have dietary caffeine intake available.

[g]The minimum lag time between measures of caffeine intake during EDIC and measures of SIF was 4 years.

[h]One hundred four subjects did not have rs1495741 genotype data available.

[i]Ln transformed.

LED, light-emitting diode.

Table 2. Association of Caffeine Intake During the Epidemiology of Diabetes Interventions and Complications Study with Skin Intrinsic Fluorescence

SIF outcome (excitation wavelength), model	*Variance*	$\beta \pm SE$	P *value*
SIF1 (375 nm)$_{kx=0.6,\ km=0.2}$[a]			
Unadjusted (M1)	11.2%	0.000321 ± 2.63E-05	2.28×10^{-32}
Adjusted (M2)	3.8%	0.000203 ± 2.43E-05	1.66×10^{-16}
SIF14 (456 nm)$_{kx=0.4,\ km=0.8}$[a]			
Unadjusted (M1)	10.7%	0.000356 ± 3E-05	7.19×10^{-31}
Adjusted (M2)	4.2%	0.000244 ± 2.98E-05	7.19×10^{-16}

The value of $\beta \pm SE$ was obtained from linear regression (*n* = 1,181). Variance was calculated as a type II squared semipartial correlation. Adjusted models included age, sex, skin tone, clinic latitude, smoking status, any estimated glomerular filtration rate of <60 mL/min/1.73 m^2, Diabetes Control and Complications Trial eligibility hemoglobinA1c, mean Diabetes Control and Complications Trial hemoglobin A1c, and mean Epidemiology of Diabetes Interventions and Complications hemoglobin A1c as covariates.

[a]Ln transformed.

M1, Model 1; M2, Model 2; SIF, skin intrinsic fluorescence.

$10^{-7} \pm 8 \times 10^{-8}$ [$P = 0.03$]) and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ ($\beta_{linear} \pm$ SE, 0.0003 ± 0.00003 [$P = 1.0 \times 10^{-16}$]; $\beta_{quadratic} \pm$SE, $-1.9 \times 10^{-7} \pm 1 \times 10^{-7}$ [$P = 0.05$]).

Caffeine intake during DCCT was consistently positively associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$, independently accounting for 2.7% and 2.3% of the variance, respectively, after adjusting for covariates in M2 (Supplementary Table S3). Mean caffeine intakes from both the DCCT and the EDIC study were independently associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ when included in the model together, with the exception for the association of mean caffeine intake during DCCT with $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ in M2 (Supplementary Table S4).

As reported previously,[10] rs1495741 was significantly associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ ($\beta \pm$ SE, -0.065 ± 0.010; $P = 6.1 \times 10^{-10}$) and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ ($\beta \pm$ SE, -0.125 ± 0.011; $P = 8.06 \times 10^{-27}$). The results were even stronger after adjusting for M2 covariates for $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ ($\beta \pm$ SE, -0.060 ± 0.008; $P = 1.7 \times 10^{-12}$) and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ ($\beta \pm$ SE, -0.123 ± 0.0096; $P = 1.4 \times 10^{-34}$). Each copy of the G allele was associated with lower SIF, explaining 3.5% and 10.1% of the variance in $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$, respectively.[10] Because NAT2 metabolizes aromatic amines found in tobacco smoke[11] and because smoking status is an important confounder, we tested whether the *NAT2* (rs1495741) effect was modified by smoking status. However, there was no significant interaction of rs1495741 with smoking status on $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ ($P = 0.13$)[10] or $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ ($P = 0.39$).

Prior to determining the joint effects of rs1495741 and mean caffeine intake during the EDIC study on SIF, we showed that rs1495741 was not associated with mean caffeine intake either during the EDIC study ($P = 0.51$) or the DCCT ($P = 0.42$). Then we showed that models containing both rs1495741 and caffeine intake during EDIC were independently associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ in fully adjusted analyses (Table 3).

To determine whether the associations for caffeine intake on SIF are due to caffeine or other constituents found in coffee, which may correlate with caffeine intake, we tested whether frequency of coffee, decaffeinated coffee, tea, and caffeinated cola (regular and low-calorie) consumption was associated with SIF. Supplementary Table S5 shows the number of subjects reporting a mean consumption of at least once per month for each beverage during EDIC years 13–15. In both Spearman correlation ($r_{SIF1} = 0.27$; $r_{SIF14} = 0.28$; $P < 0.0001$) and in adjusted regression analysis, mean coffee intake was positively associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ ($P = 8.9 \times 10^{-12}$) and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ ($P = 4.4 \times 10^{-12}$). Decaffeinated coffee consumption, however, was not associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ ($r = 0.02$; $P = 0.53$) or $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ ($r = 0.01$; $P = 0.73$) in either type of analysis, even after restricting the analysis to subjects reporting a mean consumption of >1 cup of caffeinated coffee once weekly (Spearman $r_{SIF1} = -0.03$, $P = 0.39$; $r_{SIF14} = -0.02$, $P = 0.57$).

Tea consumption was associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ in Spearman correlations ($r = 0.06$, $P = 0.04$) but not in adjusted regression analyses ($P = 0.13$).

Because 84% of subjects reported consuming regular, caffeinated cola never or less than once per month, we did not use this variable in analyses. However, low-calorie caffeinated cola in Spearman analyses was associated with $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ ($r = 0.08$, $P = 0.006$) and in covariate adjusted analyses was associated with both $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ ($P = 0.04$) and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$ ($P = 0.02$).

Finally, when including both mean coffee intake and mean caffeine consumption in the model, the effect for mean coffee intake disappears ($P = 0.19$ for $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ and $P = 0.09$ for $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$), and the positive association for caffeine consumption persists ($P = 1.9 \times 10^{-5}$ for $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$; $P = 1.3 \times 10^{-4}$ for $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$; M2 analyses).

Replication in the Pittsburgh EDC study

Subject characteristics at the time of SIF assessment are shown in Supplementary Table S6. At the time of diet assessment, mean participant age was 31 years with diabetes duration of 22 years. Caffeine was positively associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ (Spearman $r = 0.31$, $P < 0.0001$), similar to the effect size observed in the DCCT/EDIC study, accounting for 9.6% of the $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ variance in unadjusted analyses. Similar to the effect observed in the DCCT/EDIC study, rs1495741 was associated with $SIF1_{LED\ 375\ nm[0.6,\ 0.2]}$ ($\beta \pm$ SE, -0.08 ± 0.03; $P = 0.002$) and

Table 3. Association of rs1495741 and Caffeine Intake During the Epidemiology of Diabetes Interventions and Complications Study with Skin Intrinsic Fluorescence

SIF outcome (excitation wavelength), predictor	*Variance*	*β ± SE*	P *value*
SIF1 (375 nm)$_{kx=0.6,\ km=0.2}$[a]			
rs1495741	2.9%	−0.0596 ± 0.008	4.24E-13
Caffeine	3.8%	0.000203 ± 2.42E-05	1.93E-16
SIF14 (456 nm)$_{kx=0.4,\ km=0.8}$[a]			
rs1495741	9.7%	−0.12265 ± 0.009	2.98E-36
Caffeine	4.3%	0.000245 ± 2.8E-05	7.86E-18

Data shown are $\beta \pm$ SE from linear regression models including both rs1495741 and caffeine intake during Epidemiology of Diabetes Interventions and Complications effects with $SIF1_{LED\ 375\ nm[0.6,0.2]}$ and $SIF14_{LED\ 456\ nm[0.4,\ 0.8]}$, after adjusting for age, sex, skin tone, clinic latitude, smoking status, any estimated glomerular filtration rate of <60 mL/min/1.73 m², Diabetes Control and Complications Trial eligibility hemoglobin A1c, mean Diabetes Control and Complications Trial hemoglobin A1c, and mean Epidemiology of Diabetes Interventions and Complications hemoglobin A1c ($n = 1{,}077$). Variance was calculated as a type II squared semipartial correlation.

[a]Ln transformed.

LED, light-emitting diode; SIF, skin intrinsic fluorescence.

$SIF14_{LED\ 456\,nm[0.4,\,0.8]}$ ($\beta \pm SE$, -0.14 ± 0.03; $P = 1.5 \times 10^{-5}$), with each copy of the G allele associated with lower SIF. In adjusted models (Supplementary Table S7), rs1495741 was independently associated with $SIF1_{LED\ 375\,nm[0.6,\,0.2]}$, but the effect for caffeine intake was attenuated and no longer significant ($P = 0.12$). For $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$, however, both rs1495741 and caffeine intake were independently associated with $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$ (Supplementary Table S7).

Discussion

We recently identified rs1495741, near *NAT2,* as a major locus for skin fluorescence in subjects with and without diabetes.[10] Given the role of NAT2 in caffeine metabolism, we examined whether caffeine is associated with SIF, as well as whether the effect of *NAT2* (rs1495741) on SIF is independent of caffeine consumption. Here we show that caffeine contributes a proportion of the interindividual variability in SIF measures. Our findings were consistent within the DCCT/EDIC cohort using caffeine data from the DCCT and the EDIC study periods separately and replicated in an independent type 1 diabetes study. Finally, the effect of rs1495741 on SIF appears to be in part independent of caffeine consumption.

To our knowledge, this is the first study to test for an association between caffeine intake and SIF. A previous study of 147 elderly Dutch subjects examining the relationship of dietary AGEs with skin autofluorescence found no significant association between coffee consumption and skin autofluorescence.[23] Although the excitation level of $SIF1_{LED\ 375\,nm[0.6,\,0.2]}$ is similar to that used by the AGE Reader in the Dutch study (peak excitation, 360 nm) there are several reasons that may explain the discrepancy in results between the current study and the former study. First, skin collagen production is altered in the elderly[24] and therefore may result in no association observed in older subjects. Second, it is not clear how coffee intake was measured by the food habits questionnaire used, as well as whether there was any distinction between caffeinated and decaffeinated coffee consumption.[23] In the EDIC study, participants reported consumption of both decaffeinated and caffeinated coffee, and we observed a stronger association with SIF when using total caffeine intake versus caffeinated coffee consumption. Finally, The Netherlands has one of the highest consumptions of caffeine in the world,[25] and coffee intakes were higher in the Dutch study with a mean consumption of 3.4 cups/day, compared with a median of 1 cup of caffeinated coffee daily in the EDIC study. If a nonlinear caffeine–SIF effect exists, whereby there is little association at the upper end of intakes on SIF, then null associations may be observed among high caffeine consumers; however, the range of coffee intake was not stated.[23]

We showed that mean caffeine intake was associated with SIF, independent of age and smoking. Caffeine is positively correlated with age among American adults up to 64 years of age and higher in smokers.[26,27] Adjusting for both age and smoking attenuated the caffeine–SIF association as they were also positively associated with caffeine intake and SIF in the EDIC study. HbA1c was also shown to contribute to the attenuation in the relationship for mean caffeine intake during EDIC and $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$. This was likely due to the positive association of caffeine intake with HbA1c, which may result from caffeine inhibiting glucose uptake by skeletal muscle and adipocytes, decreasing postprandial glycemic control.[28] In the DCCT/EDIC study, caffeine consumption independently accounted for 4% of the variability in SIF (in M2) and, together with rs1495741 and explanatory covariates adjusted for in M2, cumulatively accounted for more than 40% of the variance in SIF.

Consistent with our study, *NAT2* has not been previously associated with coffee or caffeine intake. GWAS of coffee[29] and caffeine[30] intake have identified associations for *CYP1A2* and *AHR*, both of which are directly and indirectly involved in caffeine metabolism, respectively. Recently, six additional loci have been implicated in a GWAS of coffee consumption.[31] Furthermore, a candidate gene approach reported no nominal association of 32 single nucleotdie polymorphisms in *NAT2* with caffeine intake using gene-based tests.[30] On the other hand, *NAT2* has been associated with 1-methylxanthine:4-acetamidobutanoate, reflecting metabolites of caffeine metabolism in a GWAS of serum metabolites.[13] Similarly, a GWAS of urinary metabolite ratios identified a polymorphism in perfect linkage disequilibrium with rs1495741 to be associated with formate:succinate levels ($P = 5.1 \times 10^{-16}$).[32] Formate may reflect degradation of caffeine because NAT2 metabolizes an intermediate caffeine metabolite and produces 5-acetylamino-6-formylamino-3-methyluracil,[12] which undergoes deformylation in the urine.[33]

Given that caffeine metabolites are commonly used as a probe for NAT2 acetylation status[12,33] and that caffeine is associated with SIF, we examined whether the effects of rs1495741 and caffeine are independent. We showed that each was associated with $SIF1_{LED\ 375\,nm[0.6,\,0.2]}$ and $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$ when included in the model together, thus suggesting that the relationship is likely independent. However, because *NAT2* was not associated with caffeine intake in the DCCT/EDIC study or in previous studies[29–31] but instead has been shown to be associated with caffeine metabolite ratios,[13,34] future studies measuring caffeine metabolites are needed to more concretely examine their independent effects,[35] as well as establishing whether caffeine metabolites are associated with SIF.

Caffeine readily crosses all biological membranes,[25] and ingested caffeine has been implicated in protecting against ultraviolet B–induced skin carcinoma in epidemiological and animal studies, partly by protecting the skin as a sunscreen.[36,37] Caffeine was associated with both $SIF1_{LED\,375\,nm[0.6,\,0.2]}$ and $SIF14_{LED\,456\,nm[0.4,\,0.8]}$, which capture common fluorophores such as AGEs, but also distinct chemicals at each end of the spectrum.[2,18] Indeed, caffeine was shown to exhibit fluorescent properties with excitation/emission wavelengths in the ultraviolet range 311/363 nm,[14] but this level was not captured by $SIF1_{LED\ 375\,nm[0.6,\,0.2]}$, the lowest excitation LED examined in the DCCT/EDIC and the EDC study. Alternatively, the caffeine–SIF association we observed may reflect the effect of caffeine on photolysis of riboflavin,[38] which has excitation/emission maxima of 450/520 nm, corresponding with $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$.[39] Because caffeine can bind to riboflavin and inhibit photolysis,[38] individuals with high caffeine intake may have higher levels of riboflavin in the skin, resulting in higher skin fluorescence. Finally, we cannot rule out the possibility of the caffeine–SIF association reflecting fluorescence levels of AGEs. We addressed this by adjusting for HbA1c, and although the effect of caffeine on $SIF14_{LED\ 456\,nm[0.4,\,0.8]}$ was

attenuated, it was still significant. Further studies are needed to determine which fluorophore is responsible for the caffeine–SIF association.

Because SIF is positively associated with subclinical atherosclerosis and/or cardiovascular disease mortality,[3–7] our results suggest that adjusting for caffeine and/or coffee intake may improve prediction estimates for SIF with cardiovascular disease because in moderate amounts coffee consumption may be protective against cardiovascular disease.[26,40] Similarly, because SIF has been proposed as an alternative noninvasive screening tool in comparison with fasting glucose levels in identifying individuals with prediabetes or type 2 diabetes mellitus,[41] adjusting for caffeine and/or coffee intake may alter prediction estimates. Components other than caffeine in coffee, such as chlorogenic acid, may be protective against type 2 diabetes mellitus[42] and AGE formation,[43] and therefore adjustment for coffee or caffeine as a marker of coffee intake may be useful.

The last measure of caffeine consumption was assessed 4 years prior to SIF in the EDIC study and 8 years prior to SIF in the Pittsburgh EDC study, but despite this, the caffeine–SIF association was robust both within the DCCT/EDIC study and replicated in the EDC study. Food sources contributing to caffeine intake were only available during EDIC years 13–15, and mean intakes of decaffeinated coffee and regular soda consumption were low, somewhat limiting the power of these analyses. Future studies in populations where interindividual intakes for these beverages vary more widely are needed to confirm our observations suggesting that the caffeine–SIF association is due to caffeine and not other components highly correlated with caffeine in coffee or other caffeine food sources. Finally, studies are needed in subjects without diabetes to determine whether the association is diabetes-specific.

In conclusion, our study clearly demonstrates that caffeine is an important contributor to the interindividual variability of SIF measures in people with type 1 diabetes. The association for caffeine intake with SIF appears to be due to caffeine and/or its metabolites rather than other constituents in coffee. Finally, the effect of rs1495741 on SIF is shown to be at least in part independent of caffeine consumption. Accounting for rs1495741 and caffeine intake may alter SIF prediction estimates for risk of cardiovascular disease, prediabetes, and type 2 diabetes mellitus.

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Author Disclosure Statement

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