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Context: Advanced glycation end-products (AGEs) are thought to be involved in the pathogenesis of Alzheimer's disease. AGEs are products resulting from nonenzymatic chemical reactions between reduced sugars and proteins, which accumulate during natural aging, and their accumulation is accelerated in hyperglycemic conditions such as type 2 diabetes mellitus.

Objective: The objective of the study was to examine associations between AGEs and cognitive functions.

Design, Setting, and Participants: This study was performed as part of the Maastricht Study, a population-based cohort study in which, by design, 215 participants (28.1%) had type 2 diabetes mellitus.

Main Outcome Measures: We examined associations of skin autofluorescence (SAF) (n = 764), an overall estimate of skin AGEs, and specific plasma protein-bound AGEs (n = 781) with performance on tests for global cognitive functioning, information processing speed, verbal memory (immediate and delayed word recall), and response inhibition.

Results: After adjustment for demographics, diabetes, smoking, alcohol, waist circumference, total cholesterol/high-density lipoprotein cholesterol ratio, triglycerides, and lipid-lowering medication use, higher SAF was significantly associated with worse delayed word recall (regression coefficient, b = −0.44; *P* = .04), and response inhibition (b = 0.03; *P* = .04). After further adjustment for systolic blood pressure, cardiovascular disease, estimated glomerular filtration rate, and depression, associations were attenuated (delayed word recall, b = −0.38, *P* = .07; response inhibition, b = 0.02, *P* = .07). Higher pentosidine levels were associated with worse global cognitive functioning (b = −0.61; *P* = .04) after full adjustment, but other plasma AGEs were not. Associations did not differ between individuals with and without diabetes.

Conclusion: We found inverse associations of SAF (a noninvasive marker for tissue AGEs) with cognitive performance, which were attenuated after adjustment for vascular risk factors and depression. (***J Clin Endocrinol Metab*** **100: 951–960, 2015)**

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Abbreviations: Aβ, β-amyloid; AD, Alzheimer's disease; AGE, advanced glycation end-product; AU, arbitrary units; CEL, N(ε)-(carboxyethyl)lysine; CI, confidence interval; CML, N(ε)-(carboxymethyl)lysine; eGFR, estimated GFR; GFR, glomerular filtration rate; HbA1c, glycosylated hemoglobin A1c; HDL, high-density lipoprotein; IGM, impaired glucose metabolism; IQR, interquartile range; NGT, normal glucose tolerance; SAF, skin autofluorescence.

Dementia is highly prevalent worldwide, and the number of people with dementia is expected to increase rapidly (1). The most common cause of dementia is Alzheimer's disease (AD), which is characterized by progressive decline in cognitive function, more specifically memory loss. It is associated with the presence and the accumulation of neurofibrillary tangles and amyloid plaques in the brain (2). Although the etiology of dementia has not been elucidated, the relationship between, on the one hand, type 2 diabetes mellitus and other cardiovascular risk factors including hypertension, obesity, and smoking and, on the other hand, the development of dementia and cognitive decline has become more evident (3, 4).

Advanced glycation end-products (AGEs), which are products resulting from nonenzymatic chemical reactions between reduced sugars and proteins (5), are thought to be involved in the pathogenesis of AD (6). AGEs accumulate during natural aging, and their accumulation is accelerated in hyperglycemic conditions such as type 2 diabetes (5, 7). Long-lived proteins, including β-amyloid (Aβ), have been found to be modified by AGEs, and a recent study has shown that the formation of AGE-modified Aβ exacerbates the toxicity of Aβ (8). AGEs are present in both neurofibrillary tangles and senile plaques of patients with AD (9), and the receptor for AGE appears to be involved in the transport of amyloid peptides through the blood–brain barrier (10). In addition, AD patients with type 2 diabetes seem to have more severe AD pathology and higher AGE levels in the brain compared with those with AD alone (9).

AGEs can be measured in plasma (circulating AGEs) or estimated in tissue using a relatively simple noninvasive measurement of skin autofluorescence (SAF), a method based on the fluorescent properties of some AGEs (11). SAF has been suggested to be a simple alternative to invasive measurement of AGE accumulation and has been shown to be correlated with fluorescent (pentosidine) and even nonfluorescent plasma AGEs (N(ε)-(carboxymethyl)-lysine [CML] and N(ε)-(carboxyethyl)lysine [CEL]) in biopsy-derived skin tissue (11). In addition, SAF has recently been shown to be associated with lower gray matter volume (12), and it may therefore be hypothesized that SAF indicates AGE accumulation in other tissue, like the brain, as well. Furthermore, SAF may reflect AGE accumulation more accurately than plasma proteins because intracellular glycation is thought to be the major local source of AGEs and not all AGEs may end up in the circulation (13, 14).

Although there is evidence that AGEs might be associated with dementia and cognitive decline, research on the relationship between AGEs and cognitive function is scarce. Yaffe et al (15) have shown that the AGE pentosidine, measured in its free form in urine, was associated with a greater 9-year cognitive decline in older people independent of diabetes status. Furthermore, Chen et al (16) showed that higher levels of serum AGEs were cross-sectionally associated with mild cognitive impairment in diabetes patients. No study to date has investigated the relationship between SAF and cognitive performance on multiple cognitive domains. One recent study has shown a cross-sectional association between higher SAF and a higher likelihood of cognitive impairment, but it did not examine associations with separate cognitive functions (12).

Examination of the association between AGEs and cognitive function might provide a marker for cognitive impairment and might increase our knowledge about the etiology of cognitive decline and dementia. Therefore, the aim of our study was to examine the associations of SAF and plasma AGEs with performance on a range of cognitive tests in participants from the Maastricht Study, a population-based cohort study. Next, we investigated whether or not these associations were different in participants with and without type 2 diabetes.

Subjects and Methods

Study population

In this study, we used data from the Maastricht Study, an observational, prospective, population-based cohort study. The rationale and methodology have been described previously (17). In brief, the study focuses on the etiology, pathophysiology, complications, and comorbidities of type 2 diabetes mellitus and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals between 40 and 75 years of age living in the southern part of The Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known type 2 diabetes status for reasons of efficiency. The present report includes cross-sectional data from the first 866 participants who completed the baseline survey between November 2010 and March 2012. The examinations of each participant were performed within a time window of 3 months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Netherlands Health Council under the Dutch "Law for Population Studies" (Permit 131088–105234-PG). All participants gave written informed consent.

Skin autofluorescence

All participants were asked to refrain from smoking and caffeine at least 3 hours before the measurements. A light meal (breakfast and/or lunch), low in fat content, was allowed. SAF was measured with the AGE Reader (DiagnOptics Technologies BV). The AGE Reader is a desktop device that uses the characteristic fluorescent properties of certain AGEs to estimate the level of AGE accumulation in the skin. Technical details of this

noninvasive method have been described more extensively elsewhere (11). In short, the AGE Reader illuminates a skin surface of 4 cm^2 guarded against surrounding light, with an excitation wavelength range of 300–420 nm and a peak excitation of 370 nm. SAF was calculated as the ratio between the emission light from the skin in the wavelength range of 420–600 nm (fluorescence) and excitation light that is reflected by the skin (300–420 nm), multiplied by 100 and expressed in arbitrary units (AU). Participants were asked not to use any sunscreen or self-browning creams on their lower arms within 2 days before the measurement. SAF was measured at room temperature in a semi-dark environment while participants were at rest in a seated position. The forearm of a participant was positioned on top of the device, as described by the manufacturer. The mean of three consecutive measurements was used in the analyses. Reproducibility was assessed in 14 individuals without diabetes (six males; age, 32.2 ± 7.1 y). The intraclass correlation coefficient of three intraindividual consecutive SAF measurements was 0.83 (95% confidence interval [CI], 0.65–0.94). SAF was calculated off-line by automated analysis using AGE Reader software, version 2.3, and was observer-independent. There were no significant differences between fasting and nonfasting measurements (mean difference = 0.01 AU; $P = .73$). Reproducibility in individuals with type 2 diabetes has been evaluated previously (11) with an overall Altman error percentage of 5.03% for measurements taken over a single day. Skin pigmentation is known to influence the measurement of SAF (18). Therefore, in participants with dark-colored skin with a reflectance of 6–10%, a validated reflectance-dependent correction was made by the software (18). Measurements in participants with dark-colored skin and a mean reflectance below 6% are considered unreliable and are therefore not used to calculate SAF by the software. Therefore, these participants were automatically excluded. Additionally, a single SAF value above 10 AU was considered as unreliable; these individual measurements (n = 3) were manually excluded, and the mean of the remaining two measurements was used in analyses.

Analysis of protein-bound AGEs and lysine in plasma

Plasma AGEs were measured in EDTA samples obtained from fasting venous blood, which were stored at −80°C until analysis. Protein-bound pentosidine was quantified using HPLC with fluorescence detection, as described in detail elsewhere (19). Intra- and interassay coefficients of variation for pentosidine, as analyzed in this study, were 6.5 and 7.8%, respectively. Protein-bound CML, CEL, and lysine were quantified using UPLC MS/MS (ultraperformance) liquid chromatography-tandem MS (14). Intra- and interassay coefficients of variation were 4.5 and 6.7% for CML, 6.2 and 10.3% for CEL, and 5.0 and 5.3% for lysine. Concentrations of protein-bound pentosidine, CML, and CEL were adjusted for levels of lysine and expressed as nanomoles per millimole lysine.

Assessment of cognitive function

A concise battery (30 min) of cognitive tests was used to assess cognitive functioning (17). An a priori selection of these cognitive tests was used in the current study. Since diabetes is strongly linked to AGE accumulation, we have chosen tests that each represent cognitive domains (i.e. information processing speed, verbal memory, and executive functions) which are often used and have been shown to be most sensitive to effects of diabetes (20). Global cognitive functioning was measured by the Mini-Mental State Examination (MMSE) (score range, 0–30) (21). Verbal memory was assessed with the Visual Verbal Word Learning Test (22). In this test, 15 words are presented in five subsequent trials, followed by a recall phase immediately after each trial (immediate recall) (score range, 0–75) and a delayed recall phase 20 minutes thereafter (delayed recall) (score range, 0–15). Response inhibition was measured with the Stroop Color Word Test (23). The variable of interest was the interference measure expressed in seconds. The Letter-Digit Substitution Test (24) was used to measure information processing speed. Participants were instructed to match digits to letters as quickly as possible within 90 seconds.

Covariates

History of cardiovascular disease, diabetes duration, smoking status (never, former, current), and alcohol consumption were assessed by questionnaire (17). Participants were regarded as having a history of cardiovascular disease if they reported to have had a myocardial infarction, and/or cerebrovascular infarction or hemorrhage, and/or percutaneous artery angioplasty of/or vascular surgery on the coronary, abdominal, peripheral, or carotid arteries. Alcohol consumption was classified into three categories: nonconsumers, low consumers (≤ seven glasses per week for females and ≤ 14 glasses per week for males), and high consumers (> seven glasses per week for females and > 14 glasses per week for males). Use of lipid-lowering, antihypertensive, and glucose-lowering medication was assessed during a medication interview where generic name, dose, and frequency were registered (17). Waist circumference, glycosylated hemoglobin A1c (HbA1c), glucose levels, total and high-density lipoprotein (HDL) cholesterol, creatinine, and triglycerides were determined as described elsewhere (17). Estimated glomerular filtration rate (eGFR) was estimated according to the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation (25). Office blood pressure was determined three times on the right arm after a 10-minute rest period using a noninvasive blood pressure monitor (Omron 705IT, Omron, Japan) (17).

To determine glucose metabolism, all participants (except those who use insulin) underwent a standardized seven-point oral glucose tolerance test after an overnight fast as previously described (17). Glucose metabolism was defined according to the World Health Organization 2006 criteria as normal glucose tolerance (NGT), impaired fasting glucose, impaired glucose tolerance, and type 2 diabetes (26). Additionally, individuals without type 1 diabetes and on diabetes medication were considered as having type 2 diabetes (17). For this study, we defined having either impaired fasting glucose or impaired glucose tolerance as impaired glucose metabolism (IGM).

Level of education was assessed during the cognitive assessment and was classified into eight categories commonly used in The Netherlands (27): 1) no education; 2) primary education; 3) lower vocational education; 4) intermediate general secondary education; 5) intermediate vocational education; 6) higher general secondary education; 7) higher vocational education; and 8) university. For this study, three groups were created for educational level: low (levels 1–3), intermediate (levels 4–6), and high (levels 7 and 8). Depression was assessed by the Mini International Neuropsychiatric Interview (17,28).

Statistical analysis

Analyses were conducted using the SPSS software, version 20 for Mac OSX (SPSS Inc). Differences between tertiles of SAF were tested using ANOVA for continuous variables and χ^2 tests for categorical variables. Multiple linear regression analysis was used to estimate the association of SAF and of plasma AGEs with cognitive performance, adjusted for different sets of covariates in separate models. In model 1, we adjusted for age, which is a known predictor of AGE accumulation and cognitive performance and is therefore considered as an important potential confounder. In model 2, we added other potential important confounders: sex, educational level, and diabetes (yes/no). In model 3, we additionally adjusted for cardiovascular risk factors that have been previously associated with higher AGE accumulation and with cognitive performance, and therefore may be potential confounders (ie, smoking, alcohol, waist circumference, total cholesterol/HDL cholesterol ratio, triglycerides, and lipid-lowering medication use). Finally, in model 4, we adjusted for variables that could be potential mediators of the associations between AGEs and cognition, because they may be caused by higher AGE accumulation and may cause cognitive impairment (ie, systolic blood pressure, cardiovascular disease, depression, and eGFR). Interaction effects were tested to examine whether the association of SAF and plasma AGEs with cognitive performance differed between participants with and without diabetes. Pentosidine levels and response inhibition scores were log-transformed before regression analysis, because they were positively skewed. A P value $< .05$ was considered statistically significant in two-sided tests.

Results

Sample

Four individuals with type 1 diabetes and four participants who did not undergo cognitive assessment were excluded. Of the remaining 858 participants, we additionally excluded individuals with missing data on the independent variables SAF (n = 31) or plasma AGEs (n = 19), or on potential confounders (n = 63). This resulted in 764 individuals available for complete case analyses with SAF and 781 individuals for complete case analyses with plasma AGEs. Participants excluded due to missing values were more likely to have diabetes and use insulin, and they had higher levels of HbA1c and SAF and lower scores for cognitive performance (global cognitive functioning, information processing speed, immediate word recall, and response inhibition) ($P < .05$). There were no differences in other characteristics (data not shown).

Characteristics of the 764 participants included for analyses with SAF are shown in Table 1, stratified according to tertiles of SAF. Of these, 215 participants (28.1%) had type 2 diabetes, of whom 35 (16.3%) were newly diagnosed at study entry. Median diabetes duration was 7.0 years (interquartile range [IQR] = 3.0–11.0), and mean HbA1c level was 6.9% (SD = ±0.8). Of the 549 participants without diabetes, 126 (16.5% of the total sample) had IGM.

Mean scores for cognitive tests in the total sample were 28.9 (SD = ±1.2) for global cognitive functioning, 48.8 digits (SD = ±9.3) for information processing speed, 45.4 words (SD = ±9.7) for immediate word recall, and 9.5 words (SD = ±2.9) for delayed word recall. The median score for response inhibition was 41.7 seconds (IQR = 31.9–55.9). Participants with type 2 diabetes had significantly lower scores on all cognitive measures compared with those with NGT ($P < .001$) after adjustment for age, whereas participants with IGM did not perform significantly worse on any cognitive test compared with those with NGT ($P > .10$ for all cognitive measures). We therefore combined participants with NGT with those with IGM for the interaction analyses.

Tertiles of SAF were significantly associated with age, educational level, glucose metabolism status, smoking status, alcohol consumption, waist circumference, systolic blood pressure, history of cardiovascular disease, HbA1c level, antihypertensive medication use, lipid-lowering medication use, glucose-lowering medication use, eGFR, pentosidine level, and cognitive functions (Table 1).

SAF and cognitive performance

In unadjusted analyses, a higher SAF level was significantly associated with worse performance on all cognitive measures (regression coefficient b = −0.44, $P < .001$, for global cognitive functioning; b = −4.69, $P < .001$, for information processing speed; b = −5.28, $P < .001$, for immediate word recall; b = −1.51, $P < .001$, for delayed word recall; b = 0.11, $P < .001$, for response inhibition). After adjustment for age (Table 2, model 1), SAF was still significantly associated with all cognitive measures. After further adjustment for sex, educational level, and diabetes (Table 2, model 2), SAF was still significantly associated with immediate and delayed word recall and with response inhibition, but not with global cognitive functioning and information processing speed. After further adjustment for smoking status, alcohol consumption, waist circumference, total cholesterol/HDL cholesterol ratio, triglycerides, and lipid-lowering medication use (model 3), the association of SAF with immediate word recall was attenuated and became nonsignificant. Associations with delayed word recall and response inhibition were attenuated but remained statistically significant. Associations of SAF with delayed word recall and response inhibition were attenuated and became nonsignificant after further adjustment for systolic blood pressure, cardiovascular disease, eGFR, and depression (Table 2, model 4). In a post hoc analysis, we additionally adjusted the associations for antihypertensive medication (yes/no) use and glucose-

Table 1. Characteristics of the Study Group (n = 764), Stratified by Tertiles of SAF

Characteristic	Low	Middle	High	*P* Value
n	254	255	255	
SAF, AU, mean (SD)	2.17 (0.20)	2.65 (0.12)	3.27 (0.38)	
Age, y, mean (SD)	54.9 (8.7)	60.2 (7.3)	63.8 (6.9)	<.001
Sex, male, n (%)	129 (50.8)	138 (54.1)	154 (60.4)	.09
Educational level, low/middle/high, n (%)	26/98/130 (10.2/38.6/51.2)	31/106/118 (12.2/41.6/46.3)	66/108/81 (25.9/42.4/31.8)	<.001
Glucose metabolism status, NGT/IGM/T2DM, n (%)	175/41/38 (68.9/16.1/15.0)	153/38/64 (60.0/14.9/25.1)	95/47/113 (37.3/18.4/44.3)	<.001
Smoking status, never/former/current, n (%)	97/133/24 (38.2/52.4/9.4)	80/135/40 (31.4/52.9/15.7)	62/139/54 (24.3/54.5/21.2)	.001
Alcohol consumption, none/low/high, n (%)	39/141/74 (15.4/55.5/29.1)	34/126/95 (13.4/49.4/37.3)	57/136/62 (22.4/53.3/24.3)	.01
Waist circumference, cm, mean (SD)	95.2 (12.1)	96.0 (3.0)	100.0 (15.1)	<.001
Systolic blood pressure, mm Hg, mean (SD)	134.1 (16.9)	137.4 (19.2)	139.6 (20.1)	.01
Antihypertensive medication, n (%)	68 (26.8)	95 (37.4)	140 (55.1)	<.001
Lipid-lowering medication, n (%)	56 (22.0)	88 (34.5)	130 (51.0)	<.001
Glucose-lowering medication, none/oral[a]/insulin, n (%)	229/22/3 (90.2/8.7/1.2)	207/42/6 (81.2/16.5/2.4)	160/66/29 (62.7/25.9/11.4)	<.001
Cardiovascular disease, n (%)	31 (12.2)	36 (14.1)	70 (27.5)	<.001
HbA1c, %, mean (SD)	5.8 (0.5)	5.9 (0.7)	6.3 (0.9)	<.001
Triglycerides, mmol/L, median (IQR)	1.20 (0.82–1.73)	1.24 (0.85–1.76)	1.23 (0.88–1.88)	.59*
Total cholesterol/HDL cholesterol, mean (SD)	4.24 (1.26)	4.24 (1.24)	4.18 (1.27)	.84
eGFR, mL/min/1.73 m^2, mean (SD)	89.1 (13.84)	85.83 (13.27)	79.86 (14.15)	<.001
Depression, n (%)	8 (3.1)	9 (3.5)	14 (5.5)	.36
Pentosidine, nmol/mmol LYS, median (IQR)	0.45 (0.37–0.53)	0.47 (0.38–0.55)	0.50 (0.40–0.60)	<.001*
CML, nmol/mmol LYS, mean (SD)	74.8 (14.2)	74.6 (14.3)	73.8 (15.9)	.67
CEL, nmol/mmol LYS, mean (SD)	34.0 (10.4)	33.9 (10.4)	34.1 (10.0)	.95
Global cognitive functioning,[b] mean (SD)	29.1 (1.2)	29.0 (1.2)	28.7 (1.3)	.001
Information processing speed,[b] mean (SD)	51.5 (9.3)	48.7 (8.7)	46.1 (9.2)	<.001
Immediate word recall,[b] mean (SD)	48.0 (9.3)	46.4 (9.1)	41.8 (9.7)	<.001
Delayed word recall,[b] mean (SD)[a]	10.3 (2.8)	9.7 (2.8)	8.5 (2.9)	<.001
Response inhibition,[c] median (IQR)	37.8 (29.0–48.5)	41.1 (33.0–54.1)	48.1 (35.5–63.5)	<.001*

Abbreviations: LYS, lysine; T2DM, type 2 diabetes mellitus.

[a] Two participants in this group used glucagon-like peptide-1 receptor agonists in addition to oral glucose-lowering medication

[b] Higher scores indicate better performance.

[c] Lower scores indicate better performance.

* *P* values were derived from ANOVA with log-transformed outcomes.

Table 2. Adjusted Association Between SAF and Cognitive Performance

	b (Regression Coefficient)	95% CI	*P* Value
Global cognitive functioning[a]			
Model 1	−0.25[c]	−0.43 to −0.06	.01
Model 2	−0.10	−0.28 to 0.08	.29
Model 3	−0.08	−0.26 to 0.11	.42
Model 4	−0.08	−0.27 to 0.11	.41
Information processing speed[a]			
Model 1	−1.73	−3.03 to −0.42	.01
Model 2	−0.42	−1.68 to 0.84	.52
Model 3	−0.13	−1.42 to 1.17	.84
Model 4	0.07	−1.24 to 1.38	.91
Immediate word recall[a]			
Model 1	−2.58	−3.96 to −1.20	<.001
Model 2	−1.39	−2.68 to −0.10	.03
Model 3	−1.15	−2.46 to 0.17	.09
Model 4	−0.97	−2.30 to 0.36	.15
Delayed word recall[a]			
Model 1	−0.74	−1.16 to −0.32	.001
Model 2	−0.48	−0.88 to −0.08	.02
Model 3	−0.44	−0.85 to −0.03	.04
Model 4	−0.38	−0.79 to 0.04	.07
Response inhibition[b]			
Model 1	0.05	0.03 to 0.08	<.001
Model 2	0.03	0.00 to 0.05	.03
Model 3	0.03	0.00 to 0.05	.04
Model 4	0.02	0.00 to 0.05	.07

Model 1: Adjustment for age. Model 2: Model 1 + adjustments for sex, diabetes, and educational level. Model 3: Model 2 + adjustments for smoking, alcohol consumption, waist circumference, total cholesterol/HDL cholesterol ratio, triglycerides, and lipid-lowering medication use. Model 4: Model 3 + adjustments for systolic blood pressure, cardiovascular disease, depression, and eGFR.

[a] Higher scores indicate better performance (MMSE score for global cognitive functioning, number of digits for information processing speed, number of words for total and delayed word recall).

[b] Lower scores indicate better performance. Scores for response inhibition (seconds) are log-transformed.

[c] A regression coefficient of −0.25 indicates that one unit increase in SAF level is associated with a decrease of 0.25 points on a test for global cognitive functioning.

lowering medication use (yes/no). The association with delayed recall increased somewhat (b = −0.41; *P* = .05), whereas the association with response inhibition was somewhat attenuated (b = 0.02; *P* = .10). Other associations remained virtually unchanged (b = −0.10, *P* = .32, for global cognitive functioning; b = 0.04, *P* = .96, for speed; b = −1.08, *P* = .12, for immediate word recall).

Interactions between SAF and diabetes on cognitive measures were not significant (model 4, regression coefficient b for interaction = −0.31, *P* = .09, for global cognitive functioning; b = −0.09, *P* = .95, for speed; b = 0.41, *P* = .75, for immediate word recall; b = 0.12, *P* = .77, for delayed word recall; b = 0.02, *P* = .35, for response inhibition).

Plasma AGEs and cognitive performance

In unadjusted analyses, higher pentosidine levels were significantly associated with worse immediate and delayed word recall and response inhibition (b = −4.67, *P* = .04, for immediate word recall; b = −1.73, *P* = .01, for delayed word recall; b = 0.09, *P* = .04, for response inhibition). In adjusted models (Table 3), these associations became nonsignificant, whereas the association between pentosidine and global cognitive functioning became stronger and significant (Table 3, model 4). After additional adjustment for antihypertensive and glucose-lowering medication use, these results were largely unchanged (for global cognitive functioning, b = −0.63, *P* = .03; for speed, b = −1.46, *P* = .47; for immediate word recall, b = −3.61, *P* = .08; for delayed word recall, b = −1.11, *P* = .08; for response inhibition, b = 0.01, *P* = .72). Interactions between pentosidine and diabetes on cognitive measures were not significant (model 4, b for interaction = 0.09, *P* = .87, for global cognitive functioning; b = −3.30, *P* = .38, for information processing speed; b = −2.77, *P* = .47, for immediate word recall; b = −0.23, *P* = .85, for delayed word recall; b = 0.07, *P* = .38, for response inhibition).

Plasma CML and CEL were not significantly associated with any cognitive measures after adjustment for confounders (Supplemental Tables 1 and 2, respectively). We found no significant interactions between CML and dia-

Table 3. Adjusted Association Between Plasma Pentosidine and Cognitive Performance

	b (Regression Coefficient)	95% CI	*P* Value
Global cognitive functioning[a]			
Model 1	−0.14[c]	−0.70 to 0.42	.62
Model 2	−0.46	−1.00 to 0.07	.09
Model 3	−0.51	−1.05 to 0.04	.07
Model 4	−0.61	−1.17 to −0.04	.04
Information processing speed[a]			
Model 1	1.37	−2.56 to 5.30	.50
Model 2	−1.29	−4.98 to 2.40	.49
Model 3	−1.38	−5.15 to 2.40	.47
Model 4	−1.37	−5.30 to 2.56	.49
Immediate word recall[a]			
Model 1	−0.15	−4.33 to 4.03	.95
Model 2	−2.63	−6.40 to 1.15	.17
Model 3	−3.39	−7.23 to 0.45	.08
Model 4	−3.45	−7.45 to 0.55	.09
Delayed word recall[a]			
Model 1	−0.44	−1.71 to 0.82	.49
Model 2	−1.00	−2.18 to 0.18	.10
Model 3	−1.05	−2.25 to 0.14	.09
Model 4	−1.02	−2.26 to 0.23	.11
Response inhibition[b]			
Model 1	−0.01	−0.09 to 0.06	.74
Model 2	0.04	−0.04 to 0.11	.33
Model 3	0.03	−0.04 to 0.10	.44
Model 4	0.02	−0.06 to 0.10	.65

Model 1: Adjustment for age. Model 2: Model 1 + adjustments for sex, diabetes, and educational level. Model 3: Model 2 + adjustments for smoking, alcohol consumption, waist circumference, total cholesterol/HDL cholesterol ratio, triglycerides, and lipid-lowering medication use. Model 4: Model 3 + adjustments for systolic blood pressure, cardiovascular disease, depression, and eGFR.

[a] Higher scores indicate better performance (MMSE score for global cognitive functioning, number of digits for information processing speed, number of words for total and delayed word recall) .

[b] Lower scores indicate better performance. Scores for pentosidine and response inhibition (seconds) are log-transformed

[c] A regression coefficient of −0.14 indicates that one unit increase in log-transformed pentosidine level is associated with a decrease of 0.14 points on a test for global cognitive functioning.

betes on any of the cognitive measures (data not shown). There was a significant interaction between CEL and diabetes on global cognitive functioning (b for interaction = 0.03; $P = .003$), but not on the other cognitive measures. Stratified analyses showed that CEL was only associated with global cognitive functioning in participants without diabetes (b = −0.01; $P = .003$), but not in participants with diabetes (b = 0.01; $P = .13$).

Sensitivity analysis

Z-scores for SAF and pentosidine were calculated for each individual as their value for AGE level minus the mean and divided by the SD of the study sample. In a sensitivity analysis, we excluded participants with AGE Z-scores higher than 3 or lower than −3 to examine whether our results would change. Six participants had a Z-score higher than 3. When these six participants were excluded from analyses, the associations of SAF with immediate word recall (model 4, b = −0.99; $P = .17$) and delayed word recall did not change (model 4, b = −0.41; $P = .07$), whereas the association of SAF with response inhibition (b = 0.02; $P = .26$) was attenuated. Other associations remained nonsignificant. For analyses with pentosidine, we excluded 16 participants with Z-scores larger than 3. The association of pentosidine with global cognitive functioning (model 4, b = −0.52; $P = .15$) was attenuated and became nonsignificant. Other associations remained nonsignificant.

Discussion

This is the first study to examine the association of SAF, as an estimate of tissue AGE accumulation, and plasma AGEs with multiple cognitive functions. Our results indicate that SAF is inversely associated with memory, although cardiovascular risk factors seem to be involved in the association. In addition, we found associations between SAF and response inhibition and between pentosidine and global cognitive functioning, which should be interpreted with caution because these associations were attenuated after excluding some influential cases. There-

fore, in our study the association between SAF and memory was most robust. Because not all AGEs may end up in the circulation (13, 14), our results indicate that SAF may be a better marker for AGE accumulation in brain tissue than plasma AGEs.

The associations between SAF or plasma pentosidine and cognitive performance were not significantly different between individuals with and without type 2 diabetes. Although our study may not have enough power to detect significant differences, our results are in line with previous research demonstrating no interaction between diabetes and urinary pentosidine on cognitive decline (15).

Accumulation of AGEs in the brain has been linked to AD by increasing inflammation, oxidative stress, and subsequent neuronal dysfunction (6). These mechanisms may be involved in the development of cognitive impairment. Interestingly, in our study the strongest association was found between SAF and delayed word recall, which is the best neuropsychological predictor of AD (29). However, associations of SAF with cognitive functions were attenuated after adjustment for potential confounders/mediators. Several (cardio)vascular risk factors may confound the relationship between AGEs and cognitive impairment.

Diabetes has been associated with higher accumulation of AGEs (5) and cognitive decline (30). In addition, research has shown that obesity increases the risk of developing dementia and cognitive impairment, possibly in part through the accumulation of AGEs (31, 32). In addition, higher levels of lipids, which are involved in the formation of AGEs (33), may also increase the risk of cognitive impairment (34). In our sample, however, lipids were not associated with SAF.

In addition to in vivo production, AGEs have been found in cigarettes (35), and therefore smoking can increase AGE levels and may increase the risk of cognitive impairment (36). High alcohol consumption may increase oxidative stress, and thereby AGE levels (37), and can affect cognitive function (38). However, in our sample, high SAF level was not associated with high alcohol consumption.

Some other factors may mediate the association between AGEs and cognitive impairment, eg, systolic blood pressure, depression, kidney functioning, and cardiovascular disease. AGE accumulation may contribute to vascular stiffening, by collagen cross-linking of the vascular wall and thereby leading to (systolic) hypertension (39), which has been associated with lower cognitive performance and lower total brain matter volume (40). Additionally, depression has been associated with both vascular stiffness (41) and cognitive decline (42), but in our sample, depression was not associated with SAF. Furthermore, the kidney metabolizes and removes plasma AGEs and is a site for accumulation of AGEs. Research has shown that a decreased GFR is associated with both higher plasma AGE levels (43) and more cognitive decline (44). A decreased GFR may therefore mediate the association between AGEs and cognitive impairment but may also predict AGE accumulation. Finally, AGEs can lead to cardiovascular disease, through mechanisms discussed previously (eg, vascular stiffness and hypertension), which can in turn lead to cognitive impairment (45).

It is important to note that some of the variables we adjusted for, eg, GFR, systolic blood pressure, and cardiovascular disease, could be part of the causal pathway from AGEs to cognitive impairment. Therefore, we may have overadjusted our associations, resulting in a reduction in the potential total causal effect of AGEs on cognition by controlling for an intermediate variable (46). Moreover, because participants that were excluded from analyses (due to missing values) had higher SAF levels and lower cognitive scores, our results may be an underestimation of the true association of AGEs and cognitive functions.

Furthermore, the associations between AGE accumulation and cognition may be stronger in individuals with cognitive impairment. Studies that found associations with plasma AGEs mostly investigated this in individuals with cognitive impairment or dementia (16, 47). In addition, in one recent study, SAF was associated with cognitive impairment (score < -1.5 SD in any domain from age-, sex-, and education-adjusted norms) (12). Stronger associations could emerge in longitudinal data in which participants develop cognitive decline or impairment (15).

Strengths and limitations

Our study has several strengths. A major strength is that it is the first study to associate SAF and several plasma AGEs with separate cognitive domains. In addition, we were able to adjust for multiple important potential confounders. Our study also has some limitations. First, due to the cross-sectional design we were not able to address causal relationships. However, longitudinal data are not available yet. Second, SAF may reflect not only skin AGEs, but also non-AGE skin fluorophores (11). Nevertheless, results of previous research support the use of SAF as a marker for skin tissue AGEs (11). Third, it remains unclear whether SAF is an accurate reflectance of the level of AGE accumulation in the brain. However, research has shown that higher SAF is associated with lower brain volume (12).

Conclusion

We found significant inverse associations between SAF, a potential marker of tissue AGEs, and memory. These

were attenuated and became nonsignificant after adjustment for vascular risk factors and depression. In addition, we found associations of SAF with response inhibition and of pentosidine with global cognitive functioning, albeit not robust. Our results may suggest that AGEs are involved in the development of cognitive decline, particularly memory decline, and possibly in part through the action of vascular risk factors. More longitudinal research is needed to examine the effect of tissue and plasma AGEs on decline in separate cognitive domains.

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