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Type 2 Diabetes, Skin Autofluorescence, and Brain Atrophy

Diabetes 2015;64:279–283 | DOI: 10.2337/db14-0506

Type 2 diabetes mellitus (T2DM) is associated with brain atrophy, but the mechanisms underlying this link are unknown. Advanced glycation end products (AGEs) accumulate in T2DM, resulting in inflammation, oxidative stress, and protein cross-linking, which are known contributors to neurodegeneration. We aimed to study whether tissue AGE accumulation is associated with T2DM-related brain atrophy. We performed brain magnetic resonance imaging, cognitive tests, and noninvasive skin autofluorescence (SAF; a measure of tissue AGE levels) on people aged >55 years with and without T2DM. Multivariable linear regression was used to study the relationships among T2DM, SAF, and gray matter volume (GMV). There were 486 people included in the study. T2DM was associated with greater SAF. Greater SAF, T2DM, and cognitive impairment were each associated with lower GMV independently of age, sex, and total intracranial volume. SAF partially mediated the association between T2DM and GMV. Longitudinal studies may help confirm whether tissue AGE accumulation is associated with brain atrophy in T2DM.

Type 2 diabetes mellitus (T2DM) is associated with an increased risk of incident cognitive impairment and dementia (1). Brain atrophy may be a key driver of T2DM-related cognitive dysfunction (2). T2DM is also associated with the excessive accumulation of advanced glycation end products (AGEs) in tissues (3). AGEs are products of nonenzymatic reactions between reactive carbonyl groups of compounds (such as glucose) with proteins, lipids, or nucleic acids (4). There is a large body of evidence from in vitro model research supporting a role for AGEs in neurodegeneration and Alzheimer disease (AD) (4). Greater serum levels of AGEs are associated with cognitive decline (5) and lower gray matter volume (GMV) in older people (4). Given these observations, it is possible that AGEs play a mechanistic role in T2DM-related brain atrophy. However, there have been no previous studies examining the role of AGEs in T2DM-related brain atrophy.

Tissue AGEs can be measured reproducibly and noninvasively in the skin by means of a specialized light emitter and detector. Skin autofluorescence (SAF) measured in this manner has been shown to be highly correlated with biopsy-derived skin AGE concentrations (6). We hypothesized that SAF levels would either mediate or modify the association between T2DM and brain atrophy.

RESEARCH DESIGN AND METHODS

Sampling

We used a cross-sectional study design, and sampling methods have been described previously (2). Participants

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Received 28 March 2014 and accepted 15 July 2014.
This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db14-0506/-/DC1.

were included from two studies: the Cognition and Diabetes in Older Tasmanians study (CDOT) and the Tasmanian Study of Cognition and Gait. Participants with T2DM aged ≥55 years were recruited into CDOT between January 2008 and January 2010 using the National Diabetes Service Scheme database as a sampling frame. The Tasmanian Study of Cognition and Gait sample was recruited by mailing approach letters to eligible registrants aged ≥55 years, living in the same Southern Tasmanian postcodes as those in the CDOT study, and has been described previously (4). The phenotype of T2DM was based on self-report and confirmed using a single plasma glucose level according to standard criteria (fasting plasma glucose ≥7.0 mmol/L, random plasma glucose ≥11.1 mmol/L, and HbA_{1c} >6.5% [48 mmol/mol]). People living in a nursing home, those with insufficient English for cognitive testing, or contraindication to magnetic resonance imaging (MRI) were excluded. The Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Human Research Ethics Committee approved the study, and we obtained written, informed consent.

Measurements

SAF

We used the AGE reader (DiagnOptics BV, Groningen, the Netherlands) to measure SAF. The spectrometer reader uses a light source to illuminate ~4 cm^2 of skin on the volar surface of the right arm 10 cm below the elbow fold. SAF is calculated as the ratio between the emission light and reflected excitation light, multiplied by 100 and expressed in arbitrary units. In our laboratory, the test-retest reliability for SAF was high (intraclass correlation coefficient 0.93; n = 11) when individuals were measured 5 days apart.

MRI Scans

MRI scans were obtained using a single 1.5T General Electric scanner with the following sequences: high-resolution T1-weighted spoiled gradient echo (GRE; TR 35 ms; TE 7 ms; flip angle 35°; field of view 24 cm; 120 contiguous slices; and isotropic voxel size 1 mm^3); T2-weighted fast spin echo (repetition time [TR], 4,300 ms; echo time [TE], 120 ms; number of excitations, 1; turbo factor, 48; and voxel size, 0.90 × 0.90 × 3 mm); fluid attenuated inversion recovery (TR, 8,802 ms; TE, 130 ms; TI, 2,200 ms; and voxel size, 0.50 × 0.50 × 3 mm); and GRE (TR, 0.8 ms; TE, 0.015 ms; flip angle, 30°; and voxel size, 0.9 × 0.9 × 7 mm).

Brain Volumes

Three-dimensional T1 and axial GRE sequences were registered into standard Montreal Neurological Institute space using Functional Magnetic Resonance Imaging of the Brain's Linear Image Registration Tool. A multispectral segmentation process was applied using three-dimensional T1 and GRE sequences using Statistical Parametric Mapping software version 5 (7) to produce tissue probability maps of gray and white matter. Tissue maps were smoothed using an isotropic 8-mm Gaussian kernel. A single expert manually segmented both hippocampi using established methods known to have high test-retest reliability in our laboratory (intraclass correlation coefficient 0.97) (8). Tissue volumes of the segmented areas (total gray, white matter, and hippocampal) were calculated using standard voxel-counting algorithms.

Other Measurements

Standardized questionnaires were used to record demographic and clinical information. Weight, height, waist and hip circumferences, habitual physical activity using a pedometer worn over 1 week, and blood pressure (BP) in a sitting position as an average of three recordings from the right arm were measured and BMI calculated. A standardized cognitive battery was used to test domains of memory, speed, and executive and visuospatial function (Supplementary Table 1) as described previously (2). Diagnosis of cognitive impairment was assigned, blinded to T2DM status, if function in any of the domains was <1.5 SDs from age-, sex-, and education-adjusted norms.

Data Analysis

The analyses were conducted on a complete dataset consisting of those in whom both measures of SAF and brain imaging were available.

Logistic regression was used to describe the associations of T2DM and brain atrophy with cognitive impairment. Linear regression was used to estimate the associations of SAF and T2DM with measures of brain atrophy. Covariates for age, sex, total intracranial volume (TICV), and other variables were added to the regression models for brain atrophy if their inclusion produced a statistically significant increase in model fit or changed the coefficient of the covariate for T2DM by >10%. Putative factors considered were hypertension (defined as mean BP >140/90 mmHg or previous diagnosis), ever smoked tobacco, creatinine, mean steps per day, history of ischemic heart disease, stroke, hyperlipidemia, BMI and waist-to-hip ratio, and the use of specific medications that have been shown to influence AGE levels (pravastatin, irbesartan, and metformin) (9–11). We then examined whether SAF mediated the associations estimated between T2DM and brain atrophy. For this, we entered SAF into the model relating T2DM to brain volume outcome measures adjusting for age, sex, smoking, serum creatinine, and TICV. If the introduction of SAF substantially attenuated the regression coefficient of the binary covariate for T2DM, and the coefficient of SAF remained largely unchanged from its value without T2DM in the model, it was considered a potential mediator. We also investigated any modifying effect (interaction) of SAF using a test of significance of the coefficient of a covariate formed as the product of the covariates for T2DM and SAF. Statistical analyses were carried out using STATA version 11.1 (StataCorp, College Station, TX).

RESULTS

There were 285 people with T2DM (mean age 67.5 years, SD 6.9) and 201 in the non-T2DM comparison group (mean age 73.4 years, SD 6.9) with SAF measures. A total of seven participants had inaccurate measures of SAF and were excluded from the analysis. Summary measures of the characteristics of each group are presented in Table 1. Comparisons of the characteristics of people with and those without T2DM are presented in Supplementary Table 2.

Associations of SAF With Study Factors

Greater SAF was associated with greater age (β = 0.014; $P < 0.001$), but not with sex (β = 0.07; $P = 0.26$). After adjustment for age and sex, T2DM was associated with greater SAF (β = 0.14; $P = 0.007$). In the whole sample (T2DM and non-T2DM), greater BMI ($P < 0.001$), less habitual physical activity ($P = 0.009$), and greater serum creatinine ($P = 0.006$) were individually associated with greater SAF. Among those with T2DM, greater SAF was associated with greater HbA_{1c} ($P < 0.001$) and longer duration of T2DM ($P = 0.016$). Among those without T2DM, there was no association between SAF and HbA_{1c}.

T2DM was associated with lower GMV (standardized β = −0.020; $P = 0.05$), but not with total hippocampal volume (standardized β = 0.03; $P = 0.51$) or white matter volume (standardized β = 0.002; $P = 0.90$). Lower GMV was significantly associated with the risk of any cognitive impairment (β = −0.03; CI −0.04 to −0.01; $P = 0.005$). In the whole sample, greater levels of SAF were significantly associated with the risk of any cognitive impairment (β = 0.41; CI 0.01–0.82; $P = 0.05$) and with lower GMV (standardized β = −0.036; $P < 0.001$), but not with hippocampal volume (standardized β = −0.046; $P = 0.258$) or white matter volume (standardized β = 0.019; $P = 0.096$) independent of age, sex, smoking, serum creatinine, and TICV. Addition of SAF attenuated the association between T2DM and GMV by 20%, rendering it nonsignificant (standardized β = −0.016; $P = 0.12$), whereas SAF remained independently associated with GMV in the model (standardized β = −0.034; $P < 0.001$). Additional adjustments for BMI, HbA_{1c}, or duration of T2DM did not change these relationships (data not shown). There was no interaction between T2DM and SAF in explaining GMV. Fig. 1 shows the scatter plots of the association between SAF and GMV stratified by T2DM status.

Table 1—Sample characteristics

	Mean (SD) or *N* (%) total (*n* = 486)
Age (years)	69.9 (7.5)
Female sex	208 (43)
Diabetes	285 (59)
Formal education (years)	11.3 (3.7)
Self-reported history of hypertension or mean systolic BP >140 or mean diastolic BP >90 mmHg	374 (77)
Use of BP-lowering medications	284 (58)
Statin use	223 (46)
Ischemic heart disease	81 (17)
TIA or stroke	32 (7)
Hyperlipidemia	153 (31)
Ever smoked	253 (52)
BMI (kg/m^2)	29.1 (5.0)
Normal (BMI 20–25)	86 (18)
Overweight (BMI 25–30)	216 (44)
Obese (BMI >30)	177 (36)
Mean steps per day	6,337 (3,372)
Serum creatinine (μmol/L)	78.5 (24.7)
Any cognitive impairment	146 (30%)
SAF (AUs)	2.05 (0.53)

AU, arbitrary unit; TIA, transient ischemic attack.

DISCUSSION

This is the first study examining the relationship among tissue AGE accumulation, T2DM, and GMV. T2DM was associated with greater accumulation of tissue AGEs (as measured by SAF) and with lower GMV. Consistent with our previous study of circulating AGEs (4), we found that greater SAF was independently and modestly associated with lower GMV, but additionally demonstrate that SAF may partially mediate the association between T2DM and lower GMV. The associations we describe are novel and provide a solid basis for further studying the relationship between tissue AGE accumulation and brain atrophy.

There is strong evidence from basic science research that tissue AGE accumulation plays a role in the pathogenesis of dementia (12). In the case of AD (the most common type of dementia), autopsy studies have shown the process of atrophy is due to the accumulation of extracellular amyloid plaque and intracellular tau neurofibrillary tangles (13). Greater levels of AGEs have been found to be colocated with amyloid plaques (14,15) and paired helical filament tau in sporadic AD (16) and may act by stabilizing plaques and promoting fibrillation of tau through protein cross-linking (17,18). We speculate that SAF may reflect AGE-mediated cross-linking of other cellular proteins, such as in neurons.

AGEs may also be directly cytotoxic to neurons in culture (19) and able to directly induce inflammation and oxidation (12) by binding with receptor for AGE (RAGE) in mitochondria, generating free radicals, and reducing clearance of pre-existing reactive oxygen species (17). Furthermore, RAGE interacts with serum β-amyloid, increasing the transport of β-amyloid across the blood–brain barrier, activating proinflammatory cytokines, and reducing cerebral blood flow (20).

Strengths of our study include the large sample size, reproducible and sensitive measures of tissue AGEs and

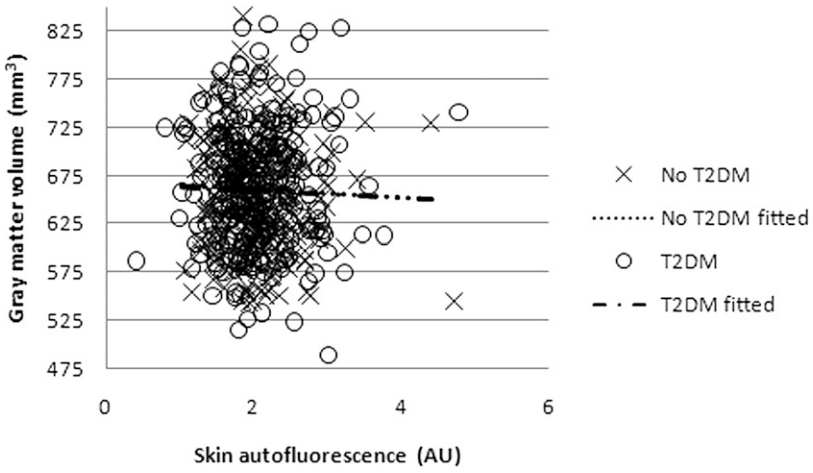

Figure 1—The association of SAF with GMV. The raw data points of the association of SAF and GMV stratified by T2DM. The fitted lines show the stratified associations between SAF and GMV adjusted for age, sex, smoking, serum creatinine, and TICV. In those without T2DM: SAF $\beta = -4.78$; standardized $\beta = -0.41$; $P = 0.003$; and adjusted $R^2 = 0.97$. In those with T2DM: SAF $\beta = -3.60$; standardized $\beta = -0.03$; $P = 0.02$; and adjusted $R^2 = 0.96$ .The high R^2 values reflect the adjustment for TICV (head size), which is collinear with GMV. The fitted lines for the two groups also overlap considerably, demonstrating a lack of interaction between T2DM and SAF in explaining GMV.

brain structure, clear definition of T2DM, and careful statistical modeling. We carefully adjusted as required for smoking, renal function, BMI, hypertension, and hyperlipidemia that may be related to both AGEs and brain or vascular health. Although medications used to treat these conditions (pravastatin and irbesartan) (9,10) and specific antidiabetes drugs such as metformin (11) have been postulated to have a protective effect against the effects of AGEs, adjusting for the use of these medications did not change our findings (data not shown). Our study has some limitations. Our study is cross-sectional, limiting inferences of causality, and needs to be confirmed in longitudinal analyses. The AGE reader does not measure AGEs that do not exhibit autofluorescence (nonfluorophores) and may also measure non-AGE fluorophores (6). However, the results of a number of earlier studies support the use of SAF as a surrogate marker of fluorescent and nonfluorescent AGE content in the skin (6,21).

Given the modest strength of β coefficients for SAF with GMV, it is likely that AGE accumulation is only one of a large number of pathways that contribute to the development of dementia in T2DM, explaining why SAF only partially mediated the T2DM–GMV relationship. The clinical relevance of these results is uncertain, but they support further research to understand the role of AGEs in the pathogenesis of dementia in relation to T2DM and overall. Prospective studies are needed to assess if tissue AGE accumulation is causally related to brain atrophy in T2DM and, subsequently, to study whether limiting AGE accumulation may slow neurodegeneration.

Funding. This study was funded by the National Health and Medical Research Council (NHMRC; project grants 403000 and 436797), Australia. C.M. is a recipient of an Alzheimer's Australia Dementia Research Foundation Scholarship and a Monash Health Clinical Academic Fellowship. G.M., R.B., and T.G.P. are recipients of NHMRC project grants. J.M.F. and A.J.V. are recipients of an NHMRC fellowship and NHMRC project grants. L.B. is supported by an NHMRC Career Development Fellowship and is a recipient of NHMRC project grants. V.S. is a recipient of a National Heart Foundation/NHMRC Career Development Fellowship and NHMRC project grants.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. C.M. drafted and revised the manuscript and performed statistical analysis and data interpretation. G.M. and J.M.F. contributed to discussion and reviewed the manuscript. R.B. performed image analysis and interpreted data. L.B. contributed to analysis and interpretation of the data and reviewed the manuscript. A.J.V. contributed to interpretation of data and reviewed the manuscript. T.G.P. supervised image analysis, contributed to discussion, and reviewed the manuscript. J.C. performed image analysis and interpreted data. V.S. developed the study concept and design, performed analysis and interpretation of data, revised the manuscript, and obtained funding. V.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol 2006;5:64–74

2. Moran C, Phan TG, Chen J, et al. Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. Diabetes Care 2013;36:4036–4042

3. Bucala R, Cerami A. Advanced glycosylation: chemistry, biology, and implications for diabetes and aging. Adv Pharmacol 1992;23:1–34

4. Srikanth V, Westcott B, Forbes J, et al. Methylglyoxal, cognitive function and cerebral atrophy in older people. J Gerontol A Biol Sci Med Sci 2013;68:68–73

5. Yaffe K, Lindquist K, Schwartz AV, et al. Advanced glycation end product level, diabetes, and accelerated cognitive aging. Neurology 2011;77:1351–1356

6. Meerwaldt R, Graaff R, Oomen PH, et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. Diabetologia 2004;47:1324–1330

7. Ashburner J, Friston KJ. Unified segmentation. Neuroimage 2005;26:839–851

8. Wrench JM, Wilson SJ, Bladin PF, Reutens DC. Hippocampal volume and depression: insights from epilepsy surgery. J Neurol Neurosurg Psychiatry 2009; 80:539–544
9. Ishibashi Y, Yamagishi S, Matsui T, et al. Pravastatin inhibits advanced glycation end products (AGEs)-induced proximal tubular cell apoptosis and injury by reducing receptor for AGEs (RAGE) level. Metabolism 2012;61:1067–1072
10. Matsui T, Yamagishi S, Takeuchi M, Ueda S, Fukami K, Okuda S. Irbesartan inhibits advanced glycation end product (AGE)-induced proximal tubular cell injury in vitro by suppressing receptor for AGEs (RAGE) expression. Pharmacol Res 2010;61:34–39
11. Ishibashi Y, Matsui T, Takeuchi M, Yamagishi S. Beneficial effects of metformin and irbesartan on advanced glycation end products (AGEs)-RAGE-induced proximal tubular cell injury. Pharmacol Res 2012;65:297–302
12. Srikanth V, Maczurek A, Phan T, et al. Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease. Neurobiol Aging 2011;32:763–777
13. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991;82:239–259
14. Vitek MP, Bhattacharya K, Glendening JM, et al. Advanced glycation end products contribute to amyloidosis in Alzheimer disease. Proc Natl Acad Sci U S A 1994;91:4766–4770
15. Smith MA, Taneda S, Richey PL, et al. Advanced Maillard reaction end products are associated with Alzheimer disease pathology. Proc Natl Acad Sci U S A 1994;91:5710–5714
16. Yan SD, Chen X, Schmidt AM, et al. Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress. Proc Natl Acad Sci U S A 1994;91:7787–7791
17. Münch G, Westcott B, Menini T, Gugliucci A. Advanced glycation end-products and their pathogenic roles in neurological disorders. Amino Acids 2012; 42:1221–1236
18. Li XH, Lv BL, Xie JZ, Liu J, Zhou XW, Wang JZ. AGEs induce Alzheimer-like tau pathology and memory deficit via RAGE-mediated GSK-3 activation. Neurobiol Aging 2012;33:1400–1410
19. Takeuchi M, Bucala R, Suzuki T, et al. Neurotoxicity of advanced glycation end-products for cultured cortical neurons. J Neuropathol Exp Neurol 2000;59:1094–1105
20. Deane R, Du Yan S, Submamaryan RK, et al. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. Nat Med 2003;9:907–913
21. Meerwaldt R, Hartog JW, Graaff R, et al. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. J Am Soc Nephrol 2005;16:3687–3693