Skin Autofluorescence and Pentosidine Are Associated With Aortic Stiffening: The Maastricht Study

Skin Autofluorescence and Pentosidine Are Associated With Aortic Stiffening

The Maastricht Study

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Abstract—Arterial stiffening, as characterized by an increase in carotid–femoral pulse-wave velocity or pulse pressure, increases the risk of cardiovascular disease, especially among individuals with type 2 diabetes mellitus. Advanced glycation end products are hypothesized to play a role in the development of arterial stiffness. Therefore, we investigated the association between skin autofluorescence, an estimate of tissue advanced glycation end products, and plasma advanced glycation end products on the one hand and arterial stiffening on the other in 862 participants of The Maastricht Study (mean age of 60 years; 45% women) with normal glucose metabolism (n=469), impaired glucose metabolism (n=140), or type 2 diabetes (n=253). Associations were analyzed with linear regression analysis and adjusted for potential confounders. We found that higher skin autofluorescence as measured by the AGE Reader and plasma pentosidine were independently associated with higher carotid–femoral pulse-wave velocity (sβ 0.10; 95% confidence interval, 0.03–0.17 and 0.10; 0.04–0.16, respectively) and central pulse pressure (sβ 0.08; 95% confidence interval 0.01–0.15 and 0.07; 0.01–0.13, respectively). The associations between skin autofluorescence and pentosidine, and carotid–femoral pulse-wave velocity were more pronounced in individuals with type 2 diabetes mellitus (*P*-interaction<0.10). These results support the hypothesis that accumulation of advanced glycation end products is involved in arterial stiffening and may explain part of the increased risk of cardiovascular disease in individuals with type 2 diabetes mellitus. **(*Hypertension*. 2016;68:956-963. DOI: 10.1161/HYPERTENSIONAHA.116.07446.)** • **Online Data Supplement**

Key Words: advanced glycation end products ■ arterial stiffness ■ autofluorescence imaging ■ blood pressure ■ diabetes mellitus, type 2 ■ pentosidine

Arterial stiffening, a measure of subclinical arterial injury, is associated with cardiovascular disease (CVD) and mortality[1–4] in a variety of populations, including in type 2 diabetes mellitus (T2DM).[5] Moreover, the age-related increase in arterial stiffness is steeper in individuals with T2DM compared with individuals without.[6,7] The exact mechanisms behind the development of arterial stiffness and subsequent CVD are not completely understood. The increased accumulation of advanced glycation end products (AGEs) on long-lived proteins, such as collagen in the arterial wall, may lead to the formation of cross links, and in the arterial wall, may subsequently lead to increased stiffening.[8] Additionally, AGEs have been linked to arterial stiffness via other mechanisms like intracellular protein glycation or receptor for AGE (RAGE) activation.[9] AGEs are thus thought to play a crucial role in the development of arterial stiffness, especially in T2DM.

Indeed, several studies found an association between measures of AGE accumulation and arterial stiffening.[10–20] Only one case–control study investigated this association in individuals with T2DM. This study found no association between serum pentosidine and heart-brachial pulse-wave velocity or brachial-ankle pulse-wave velocity after adjustment for renal function.[15] However, it did not take other potential confounders into account. Moreover, the measurement of skin autofluorescence (SAF) has recently emerged as an estimate of AGE accumulation in skin tissue[21] and may thereby be a better estimate of tissue AGE accumulation than plasma AGEs. To date, no study has investigated the association between SAF and plasma AGEs and

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carotid to femoral pulse-wave velocity (cfPWV) or pulse pressure, both measures of aortic stiffening, in a population-based setting, including individuals with normal glucose metabolism (NGM), impaired glucose metabolism (IGM), and T2DM.

In view of these considerations, the aims of our study were, first, to evaluate the independent association between SAF and plasma AGEs on the one hand and measures of arterial stiffening, ie, cfPWV, central pulse pressure, and 24-hour ambulatory pulse pressure, on the other. Second, to examine whether these associations differed between individuals with NGM, IGM, or T2DM.

Methods

Study Population and Design

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.[22] In brief, the study focuses on the cause, pathophysiology, complications, and comorbidities of type 2 diabetes mellitus (T2DM) and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and 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 T2DM 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. From the initial 866 individuals in included in this study, we excluded individuals with type 1 diabetes mellitus (n=4).

Skin Autofluorescence

All participants were asked to refrain from smoking and caffeine at least 3 hours before the measurements. A light meal (breakfast or lunch), low in fat content, was allowed. SAF was measured with the AGE Reader (DiagnOptics Technologies BV, Groningen, The Netherlands). 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.[21] In short, the AGE Reader illuminates a skin surface of 4 cm^2 guarded against surrounding light, with an excitation wavelength range of 300 to 420 nm, with 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 to 600 nm (fluorescence) and excitation light that is reflected by the skin (300–420 nm), multiplied by 100 and expressed in arbitrary units. 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, whereas participants were at rest in a seated position. The inner side of the forearm ≈4 cm below the elbow fold of a participant was positioned on top of the device, as described by the manufacturer. The mean of 3 consecutive measurements was used in the analyses. Reproducibility was assessed in 14 individuals without diabetes mellitus (6 men; 32.2±7.1 years). The intraclass correlation coefficient of 3 intraindividual consecutive SAF measurements was 0.83 (95% confidence interval [CI], 0.65–0.94). SAF was calculated offline 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 arbitrary units; P=0.73). Reproducibility in individuals with T2DM has been evaluated previously[21] 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.[23] Therefore, in participants with dark-colored skin with a reflectance of 6% to 10%, a validated reflectance-dependent correction was made by the software.[23] 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 (n=1). Additionally, a single SAF value above 10 arbitrary units was considered as unreliable; these individual measurements (n=3) were manually excluded, and the mean of the remaining 2 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 high-performance liquid chromatography with fluorescence detection, as described in detail elsewhere.[24] Intra- and interassay coefficients of variation, as analyzed in this study, were 6.5% and 7.8% for pentosidine, respectively. Protein-bound Nε-(carboxymethyl)lysine (CML) and Nε-(carboxyethyl)lysine (CEL) and lysine were quantified using ultra-performance liquid chromatography tandem mass-spectrometry.[25] 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 nmol/mmol lysine.

Carotid to Femoral Pulse-Wave Velocity and Central Pulse Pressure

As described previously,[26] cfPWV and central pulse pressure (cPP) were assessed noninvasively by means of applanation tonometry. All measurements (≈45 minutes) were done by trained vascular technicians unaware of the participants' clinical or diabetes mellitus status. Measurements took place in a quiet, temperature-controlled room (21–23°C) and were performed in supine position, after 10 minutes of rest. Participants were asked to refrain from smoking and drinking coffee or tea or alcohol beverages 3 hours before the study. Participants were allowed to have a light meal (breakfast and lunch). Talking or sleeping was not allowed during the examination. A 3-lead ECG was recorded continuously during the measurements to facilitate automatic signal processing. In addition, brachial systolic, diastolic, and mean arterial pressure were determined repeatedly with a 5-minute interval, using an oscillometric device (Accutorr Plus, Datascope, Inc, Montvale, NJ), and the average of these measurements was calculated. cfPWV was determined according to recent guidelines[27] with the use of applanation tonometry (SphygmoCor, Atcor Medical, Sydney, Australia). Pressure waveforms were determined at the right common carotid and right common femoral arteries. The difference in the time of pulse arrival from the R-wave of the ECG between the 2 sites (transit time) was determined with the intersecting tangents algorithm. The pulse wave travel distance was calculated as 80% of the direct straight distance (measured with an infantometer) between the 2 arterial sites. cfPWV was defined as travelled distance/transit time. cPP was determined by radial applanation tonometry (Sphygmocor, Atcor Medical, Australia).[28] The median of 3 consecutive cfPWV and cPP recordings were used in the analyses.

Glucose Metabolism Status

As described previously,[22] to determine glucose metabolism, all participants (except those who used insulin) underwent a standardized 7-point oral glucose tolerance test after an overnight fast. Blood samples were taken at baseline and at 15, 30, 45, 60, 90, and 120 minutes after ingestion of a 75 g glucose drink. For safety reasons, participants with a fasting glucose level above 11.0 mmol/L, as determined by a finger prick, did not undergo the oral glucose tolerance test. For these individuals (n=13), fasting glucose level and information about diabetes mellitus medication use were used to determine glucose metabolism status. Glucose metabolism was defined according to the WHO 2006 criteria into normal glucose tolerance, impaired fasting

glucose, impaired glucose tolerance, and T2DM.[29] For this study, we defined having either impaired fasting glucose or impaired glucose tolerance as IGM.

For method description on 24-hour ambulatory blood pressure, peripheral neuropathy, diabetic nephropathy, and other covariates, we refer to the online-only Data Supplement.

Statistical Methods

Analyses were conducted using SPSS version 21 for Windows. Comparisons of baseline characteristics between groups were made by use of ANOVA or χ^2 tests. Variables with a skewed distribution were $\log_{10}$ transformed before analysis. We used standardized multiple linear regression analysis to evaluate the association between SAF and plasma AGEs on the one hand and cfPWV, cPP, and 24-hour ambulatory pulse pressure (aPP) on the other, which enabled us to adjust for possible confounding factors. As presented in each table, we included all available data in the analyses to avoid selection bias. We investigated whether or not these associations differed between individuals with different glucose metabolism status by adding interaction terms in our models (eg, the product of (1) SAF or plasma AGE levels and (2) glucose metabolism status). *P* values <0.05 were considered statistically significant, except for interaction terms, where a *P* value <0.10 was considered statistically significant.

Results

General Characteristics

Table 1 shows the general characteristics, stratified according to tertiles of cfPWV. Data on SAF were available in 831 individuals, plasma AGEs in 843, cfPWV data in 820, and cPP data in 828 individuals. Missing data were predominantly caused by device nonavailability or technical problems. Plasma AGEs were measured in blood samples, which, for the missing cases, were mostly not available because of difficulties in blood withdrawal. As a sensitivity analysis, we additionally performed all analyses in a data set where missing values were imputed by multiple imputation using SPSS. These additional analyses were not materially different from our original analyses. The percentage of individuals with IGM or T2DM, peripheral sensory neuropathy, albuminuria, hypertension, antihypertensive or lipid-modifying or diabetes medication, and a history of CVD was higher in the higher cfPWV tertiles. Also, with higher cfPWV tertile, the percentage of men and former smokers was higher, as were age, diabetes mellitus duration, glycohemoglobin, waist circumference, total-to-high-density lipoprotein cholesterol ratio and triglycerides; eGFR was lower with higher cfPWV. SAF and plasma pentosidine level were higher with higher cfPWV, as were mean arterial pressure and heart rate (Table 1). For the general characteristics stratified by glucose metabolism status, please see Table S1 in the online-only Data Supplement.

Associations Between AGE Accumulation and Aortic Stiffening

Both SAF (sβ 0.09; 95% CI, 0.03–0.16) and plasma pentosidine (sβ 0.09; 95% CI, 0.03–0.16) were significantly associated with higher cfPWV, after adjustment for age, sex, glucose metabolism status, average mean arterial pressure, and heart rate obtained during cfPWV measurement; waist circumference; smoking; antihypertensive, lipid-modifying, and diabetes mellitus medication use; eGFR; total-to-high-density lipoprotein cholesterol ratio; triglycerides; and a history of cardiovascular disease (Table 2). These associations were more pronounced in individuals with T2DM (SAF: sβ 0.13; 95% CI, −0.01 to 0.28; pentosidine: sβ 0.12; 95% CI, -0.02 to 0.26, *P* alues for interaction <0.10) (Figure). The association between SAF and cfPWV was also more pronounced in individuals with IGM compared to those with NGM (sβ 0.13, 95%-CI -0.05 to 0.31), but without significant interaction (*P*=0.322; Figure). SAF (sβ 0.08; 95% CI; 0.01–0.15) and plasma pentosidine (sβ 0.07; 95% CI, 0.01–0.13) showed positive and similar significant associations with cPP after adjustment for age, sex, glucose metabolism status, average 24-hour mean arterial pressure, average 24-hour heart rate, waist circumference, smoking, antihypertensive or lipid-modifying or diabetes medication use, eGFR, total-to-high-density lipoprotein cholesterol ratio, triglycerides, and a history of cardiovascular disease (Table 2). The associations between SAF and AGEs on the one hand and cPP on the other were not different for different glucose metabolism status (*P* for interaction >0.10; Figure). SAF (sβ 0.06; 95% CI, −0.01 to 0.12), plasma pentosidine (sβ 0.05; 95% CI, −0.01 to 0.11), and plasma CML (sβ 0.06; 95% CI, −0.01 to 0.12) showed positive, nonsignificant associations with aPP (Table S2).

Influence of Previous CVD, Peripheral Neuropathy, and Diabetic Nephropathy on the Associations Between AGE Accumulation and Aortic Stiffening

The inclusion of individuals with previous CVD in our analysis may have influenced the observed associations between measures of AGE accumulation and arterial stiffness. However, exclusion of individuals with previous CVD did not materially change the associations between SAF and plasma AGEs on the one hand and cfPWV and cPP on the other (Figure S1). Furthermore, additional adjustment for PSN and albuminuria, as markers of microvascular disease, did not materially change the associations between SAF and plasma AGEs on the one hand and cfPWV and cPP on the other (Table S3).

Discussion

This study had 3 main findings. First, we found that higher SAF and plasma pentosidine were independently associated with higher cfPWV and cPP. Second, in analyses stratified for glucose metabolism status, we found that the associations between SAF and plasma pentosidine on the one hand and cfPWV on the other were more pronounced in individuals with T2DM. This is the first population-based study that describes an association between SAF and measures of aortic stiffening in individuals with NGM, IGM, and T2DM. Our results are in agreement with previous studies, which demonstrated that skin AGEs are associated with arterial stiffening in type 1 diabetes mellitus,[16] heart disease, and [10] end-stage renal disease[11] and in the elderly.[12,19] We found relatively small standardized regression coefficients in these associations. In comparison, sβs and 95% CIs of the associations between mean arterial pressure and age on the one hand and cfPWV on the other were 0.37 (0.31–0.43) and 0.31 (0.24–0.38), respectively, in our study. However, the fact that these coefficients were statistically significant suggests that the association between the different measures of AGEs and measures of arterial stiffness, albeit small, indeed reflect a true association

Table 1. General Characteristics of the Maastricht Study Participants

Characteristics	Tertiles of Carotid to Femoral Pulse-Wave Velocity (n=820)			
	First Tertile (n=273); (4.8–7.8 m/s)	Second Tertile (n=274); (7.8–9.4 m/s)	Third Tertile (n=273); (9.4–20.2 m/s)	*P* Value
NGM/IGM/T2DM, %	72/11/17	57/18/25	35/20/45	<0.001
Age, y	55±8	60±8	65±6	<0.001
Sex (number of men/women)	122/151	159/115	165/108	<0.001
Diabetes mellitus duration, y	4 (2–7)	7 (2–11)	8 (5–13)	<0.001
HbA1c, %	5.8±0.7	5.9±0.6	6.3±1.0	<0.001
HbA1c, mmol/mol	40±8	41±7	45±11	<0.001
Smoking, never/former/current, %	37/45/18	28/56/16	29/57/14	0.052
Waist circumference, cm	93±13	97±13	100±13	<0.001
Total-to-HDL cholesterol ratio	4.0±1.2	4.4±1.2	4.2±1.3	0.006
Triglycerides, mmol/L	1.1 (0.8–1.6)	1.2 (0.9–1.8)	1.4 (1.0–2.0)	<0.001
$eGFR_{CKD-EPI}$, mL/min/1.73 m2	90±13	84±14	80±15	<0.001
Albuminuria (normo/micro/macro), %	95/4/1	94/5/1	88/11/1	0.008
Peripheral sensory neuropathy, n (%)	9 (4)	16 (8)	40 (22)	<0.001
Hypertension, n (%)	97 (36)	157 (57)	217 (80)	<0.001
Antihypertensive medication, n (%)	74 (27)	108 (39)	141 (52)	<0.001
Lipid-modifying medication, n (%)	68 (25)	92 (34)	136 (50)	<0.001
Diabetes mellitus medication, n (%)	41 (15)	57 (21)	88 (32)	<0.001
History of CVD, n (%)	33 (13)	46 (17)	60 (24)	0.003
SAF, AU	2.55±0.51	2.70±0.50	2.88±0.55	<0.001
Pentosidine, nmol/mmol LYS	0.44 (0.37–0.53)	0.47 (0.38–0.56)	0.49 (0.40–0.61)	<0.001
CML, nmol/mmol LYS	74.8±14.4	74.7±14.7	73.6±15.1	0.586
CEL, nmol/mmol LYS	34.3±10.8	33.2±9.3	34.8±10.6	0.183
cfPWV, m/s	6.98±0.59	8.60±0.47	11.4±1.8	…
MAP, mm Hg	92±8	98±9	103±10	<0.001
Heart rate, bpm	62±8	63±8	65±9	<0.001
Central pulse pressure, mm Hg	38 (33–47)	45 (38–55)	55 (44–68)	<0.001
Ambulatory 24-h PP, mm Hg	41±7	44±8	51±9	<0.001
Ambulatory 24-h MAP, mm Hg	90±7	93±8	96±9	<0.001
Ambulatory 24-h heart rate, bpm	70±8	69±9	71±9	0.014

Data are presented as mean±SD or as median (IQR), unless otherwise indicated. AU indicates arbitrary units; CEL, N(epsilon)-(carboxyethyl)lysine; CML, N(epsilon)-(carboxymethyl)lysine; cfPWV, carotid to femoral pulse-wave velocity; CVD, cardiovascular disease; DBP, diastolic blood pressure; $eGFR_{CKD-EPI}$, estimated glomerular filtration rate; HbA1c, glycohemoglobin; HDL, high-density lipoprotein; IGM, impaired glucose metabolism; MAP, mean arterial pressure; NGM, normal glucose metabolism; SAF, skin autofluorescence; SBP, systolic blood pressure; and T2DM, type 2 diabetes mellitus.

between AGE accumulation and aortic stiffening. In fact, the effect size on an association does not necessarily reflect the biological importance of the pathophysiological process. Therefore, these results provide us with more insight into the pathophysiology of arterial stiffening and the possible role of AGEs herein.

AGEs are thought to affect vascular tissue via distinct pathways. First, certain AGEs, for example, pentosidine, are able to form cross-links between extracellular matrix proteins such as collagen in the arterial wall, which may directly result in a decrease in vascular elasticity and an increase in arterial stiffening.[8,13,30] Second, other AGEs, for example, CEL, are able to affect cell function via intracellular glycation of proteins, altering the function of these proteins,[9] for example, leading to the quenching of nitric oxide resulting in increased smooth muscle cell tone, which contributes to arterial stiffening.[31] Third, some AGEs, for example, CML, are known to bind to the RAGE, inducing receptor-mediated cell activation and low-grade inflammation,[32,33] which in its turn may promote arterial stiffening via for example, MMPs, endothelial dysfunction that elevates smooth muscle tone, and a reduction of endothelial flow–mediated dilation.[31] In addition to an association between SAF and measures of aortic stiffening, we found a positive association between plasma pentosidine and aortic stiffening.

Table 2. Associations Between Measures of AGE Accumulation and cfPWV or cPP

AGEs	Model	Carotid to Femoral Pulse-Wave Velocity			Central Pulse Pressure		
		sβ	95% CI	*P* Value	sβ	95% CI	*P* Value
SAF	1	0.10	0.03 to 0.17	0.006	0.06	−0.02 to 0.14	0.114
	2	0.11	0.05 to 0.17	0.001	0.07	0.00 to 0.14	0.050
	3	0.10	0.03 to 0.17	0.004	0.08	0.01 to 0.15	0.018
Plasma pentosidine	1	0.08	0.02 to 0.15	0.011	0.09	0.03 to 0.16	0.007
	2	0.08	0.02 to 0.14	0.005	0.09	0.03 to 0.15	0.003
	3	0.10	0.04 to 0.16	0.002	0.07	0.01 to 0.13	0.025
Plasma CML	1	−0.01	−0.07 to 0.06	0.870	0.05	−0.02 to 0.12	0.135
	2	0.00	−0.06 to 0.06	0.928	0.06	0.00 to 0.12	0.062
	3	0.00	−0.06 to 0.07	0.895	0.04	−0.03 to 0.10	0.281
Plasma CEL	1	0.03	−0.04 to 0.09	0.393	−0.02	−0.09 to 0.05	0.561
	2	0.03	−0.03 to 0.08	0.348	−0.02	−0.08 to 0.04	0.556
	3	0.01	−0.04 to 0.07	0.643	0.00	−0.06 to 0.06	0.932

Model 1 is adjusted for age, sex, and glucose metabolism status. Model 2 is additionally adjusted for average mean arterial pressure. Model 3 is additionally adjusted for average heart rate, waist circumference, smoking, antihypertensive and lipid-modifying and diabetes medication use, eGFR, total-to-HDL cholesterol ratio, triglycerides, and a history of cardiovascular disease. There were 740 individuals (414 with NGM, 122 with IGM, and 203 with T2DM) included in the analyses between SAF and cfPWV, 752 individuals (417 with NGM, 122 with IGM, and 208 with T2DM) in the analyses between plasma AGEs and cfPWV, 726 individuals (401 with NGM, 127 with IGM, and 208 with T2DM) included in the analyses between SAF and cPP and 739 (405 with NGM, 127 with IGM, and 207 with T2DM) individuals in the analyses between plasma AGEs and cPP. Standardized β, the standardized regression coefficient obtained with linear regression analyses, indicates the change in cfPWV or cPP (in SD) per 1 SD higher SAF or level of plasma advanced glycation endproducts, that is, plasma pentosidine, CML and CEL. AGE indicates advanced glycation end product; CEL, N(epsilon)-(carboxyethyl)lysine; cfPWV, carotid to femoral pulse-wave velocity; CI indicates confidence interval; CML, N(epsilon)-(carboxymethyl) lysine; cPP, central pulse pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IGM, impaired glucose metabolism; NGM, normal glucose metabolism; SAF, skin autofluorescence; and T2DM, type 2 diabetes mellitus

One previous study found no association between serum pentosidine and heart-brachial PWV or brachial-ankle PWV after adjustment for renal function.[15] However, this was based on a small, case–control study. Next, we found no association between plasma CML and CEL and cfPWV or cPP. This is in contrast with other studies that demonstrated an association between the plasma AGEs, CML, and CEL, and arterial stiffening in other populations.[13,14,17–19] In the case of CML, it has recently been described that the trapping of CML by the RAGE in adipose tissue causes a decrease in AGE plasma levels in individuals with T2DM.[34] Therefore, plasma CML may not be a good reflection of CML accumulation in tissues in individuals with T2DM. This may explain why we did not find an association between plasma CML and aortic stiffening in our population. We do not have a clear explanation why we found no association between CEL and aortic stiffening, whereas others, in different populations, did. Because we only found an association between plasma pentosidine and cfPWV and cPP, this could indicate that cross-linking of AGEs, and not, or to a lesser extent intracellular glycation or RAGE activation, is the predominant pathway through which AGEs lead to aortic stiffening in T2DM. However, we cannot exclude the possibility that plasma pentosidine is simply a better reflection of the detrimental effects of AGEs on the vessel wall in general. Taken together, the results of our study combined with previous research, support the hypothesis that AGE accumulation, and in particular AGE cross-linking, may play a role in the development of arterial stiffening in individuals with NGM, IGM, and T2DM. We found nonsignificant positive associations between SAF, plasma pentosidine, and CML on the one hand and aPP on the other. These results may be explained by the fact that cfPWV, as the gold-standard measurement of aortic stiffening,[28] and cPP are more precise markers of arterial stiffening compared with aPP. The associations between both SAF and pentosidine on the one hand and cfPWV on the other were more pronounced in individuals with T2DM. Additionally, the association between SAF and cfPWV was also more pronounced in individuals with IGM. This could be caused by the fact that in individuals with higher AGE levels, there is not only more cross-linking but also more RAGE activation with subsequent low-grade inflammation, and more intracellular glycation. As discussed above, these mechanisms could both lead to a further increase in arterial stiffening. Additionally, we cannot exclude the possibility that for individuals with NGM, having less variation in AGE accumulation and arterial stiffening compared with individuals with T2DM, it is more difficult to find an association between the 2. We excluded the possibility that the inclusion of individuals with previous CVD or microvascular disease influenced our findings, by repeating analyses in individuals without CVD only and by adjusting for markers of microvascular disease, after which we found similar results. When we additionally adjust the association between SAF and plasma AGEs on the one hand and cfPWV on the other for fasting glucose level, we find smaller sβ (sβ 0.06; 95% CI, −0.01 to 0.14 for SAF, and sβ 0.05; 95% CI, −0.02 to 0.11 for pentosidine), and associations were no longer significant. This could mean that part of the association between AGE measurements and arterial stiffening is explained by a difference in glucose

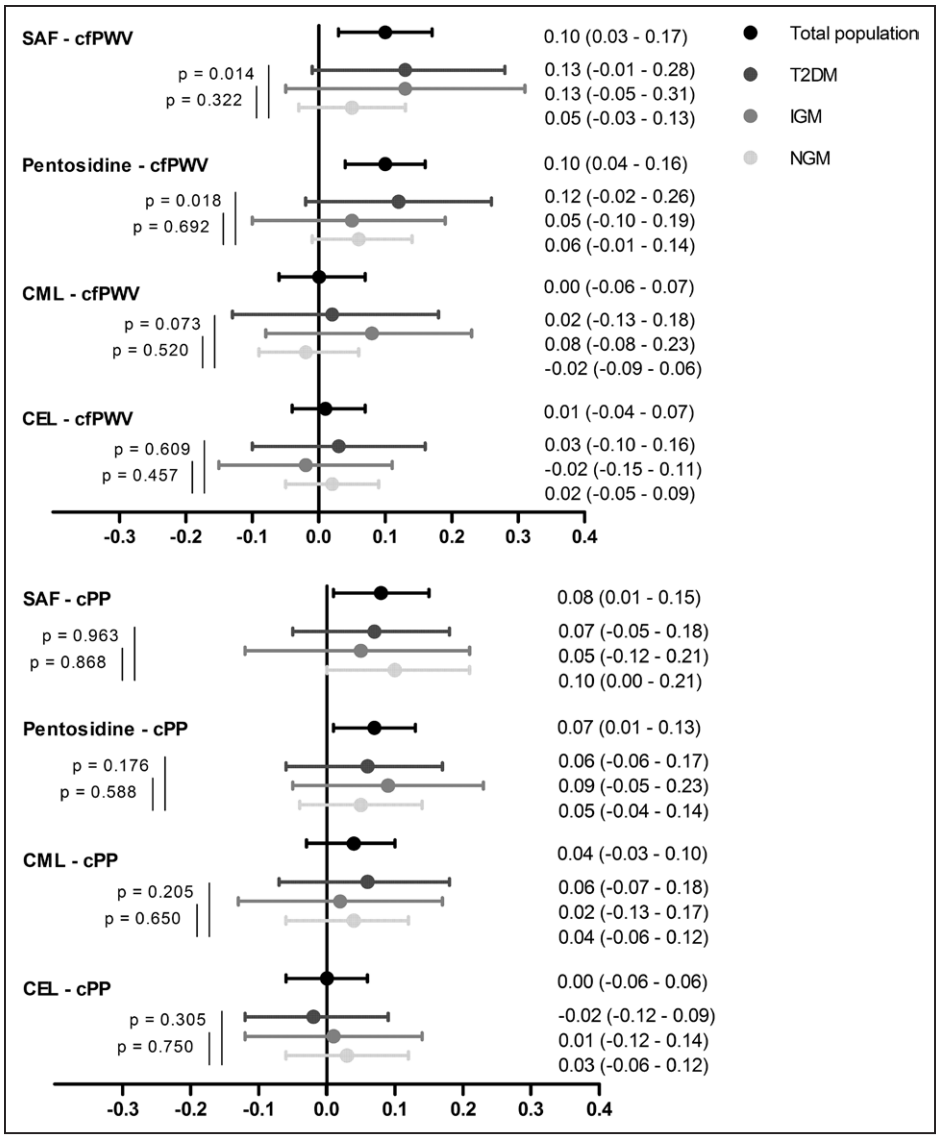

Figure. Associations between measures of advanced glycation end product (AGE) accumulation and carotid to femoral pulse-wave velocity (cfPWV) or central pulse pressure (cPP) in individuals with normal glucose metabolism (NGM), impaired glucose metabolism (IGM), and type 2 diabetes mellitus (T2DM). Data are presented as standardized β (sβ) and 95% confidence interval. sβ is the standardized regression coefficient obtained with linear regression analyses, which indicates the change in cfPWV or cPP in SD per 1 SD higher skin autofluorescence (SAF), level of plasma pentosidine, plasma Nε-(carboxymethyl)lysine (CML) or plasma Nε-(carboxyethyl) lysine (CEL) in the total population, individuals with normal glucose metabolism (NGM), impaired glucose metabolism (IGM), or T2DM. The presented sβs are adjusted for age and sex, average mean arterial pressure and heart rate, waist circumference, smoking, antihypertensive and lipid-modifying and diabetes mellitus medication use, estimated glomerular filtration rate, total-to-high-density lipoprotein cholesterol ratio, triglycerides, and a history of cardiovascular disease. The displayed *P* values indicate the significance of interaction of glucose metabolism status in these associations.

levels between GMS groups. Another explanation for these findings is that glucose is a marker in the same pathway as SAF and AGEs, which causes the regression coefficient to diminish. For this study, we used both SAF, an estimate of skin AGEs, and protein-bound AGEs in plasma to serve as a reflection of tissue AGEs. SAF is thought to reflect AGE accumulation and AGE cross-linking in the extracellular matrix of the vessel wall more accurately than plasma proteins, as plasma AGE levels are determined to a large extent by the half-life of plasma proteins, which is significantly shorter than the half-life of long-lived proteins in the skin and in vascular tissue.[35] The fact that we found an association between SAF and aortic stiffening and not between plasma CML or CEL and aortic stiffening further supports this hypothesis, at least for plasma CML and CEL. As AGE accumulation may be involved in the development of arterial stiffening and CVD in individuals with T2DM, AGE-lowering therapies may decrease the risk of CVD in individuals with T2DM. One of the well-studied, potential, anti-AGE therapies is the cross-link breaker alagebrium (ALT-711). Indeed, it has been shown that alagebrium is able to reduce large artery stiffening in animal models.[36] One double-blind randomized controlled trial correspondingly showed a decrease in pulse pressure and cfPWV in individuals who received alagebrium.[37] However, another double-blind randomized controlled trial

showed no treatment effects on cardiac function and exercise tolerance.[38]

Strengths of this study include the large and well-characterized samples, the assessment of aortic stiffening and the use of state-of-the art techniques to measure multiple markers of glycation end products. Limitations of the study include, first, this cross-sectional design of the study; therefore, we cannot draw any conclusions about causality in the association between AGE accumulation and aortic stiffening. Second, by stratifying for glucose metabolism status, we performed analyses in a smaller number of individuals, especially in the IGM group, which diminishes the power to detect an association. Third, as stated previously, we do not know whether and to what extent the different plasma AGEs reflect specific pathophysiological pathways, that is, cross-linking, intracellular protein glycation, or RAGE activation, or are merely a reflection of AGE formation and vascular damage in general.

Perspectives

Arterial stiffening increases the risk of cardiovascular disease, especially among individuals with type 2 diabetes mellitus. We demonstrate that higher levels of SAF, plasma pentosidine, and plasma CML were associated with more aortic stiffening and that associations for cfPWV were more pronounced in individuals with T2DM. These results support the hypothesis that AGE accumulation is involved in arterial stiffening in general, and, moreover, the accelerated arterial stiffening in individuals with T2DM. Prospective studies are needed. Assuming causality between AGEs and arterial stiffness, interference in the pathways of AGE accumulation might influence the development and progression of arterial stiffening, in particular in individuals with T2DM. Therefore, more large, specific, and well-designed studies are needed to elucidate their potential effect in humans, in particular individuals with T2DM.

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Disclosures

A.J. Smit is founder and shareholder of Diagnoptics Technologies BV, The Netherlands, manufacturing AGE readers (http://www.diagnoptics.com). The other authors report no conflicts.

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Novelty and Significance

What Is New?

- This study demonstrates in a population-based setting that higher levels of advanced glycation end products (AGEs), as measured with state-of-the art techniques, were associated with more aortic stiffening and that this association was more pronounced in individuals with type 2 diabetes mellitus.

What Is Relevant?

- Assuming causality between AGEs and arterial stiffening, interference in AGE accumulation may influence the development and progression of arterial stiffening, in particular in individuals with type 2 diabetes mellitus.

Summary

AGEs were associated with more aortic stiffening. These data argued in favor of studying AGE-lowering therapies to decrease arterial stiffening and the risk of cardiovascular disease.