Plasma Levels of Advanced Glycation End Products Are Related to the Clinical Presentation and Angiographic Severity of Symptomatic Lower Extremity Peripheral Arterial Disease

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Abstract

Evidence implicates a role of advanced glycation end products (AGEs) in the development of atherosclerosis. The present study examined the relationship between plasma levels of AGEs and the clinical and angiographic characteristics of patients with symptomatic peripheral arterial disease (PAD). A total of 40 consecutive patients with symptomatic lower extremity PAD undergoing invasive evaluation were enrolled. Clinical history, angiographic data, and plasma levels of total AGE (tAGE), N-carboxymethyllysine (CML), and high-sensitivity C-reactive protein were obtained. In multivariate analyses, there were independent relationships noted between tAGE levels and the presence of critical limb ischemia (CLI) ($r^2 = 0.195$, $p = 0.003$), Rutherford stage ($r^2 = 0.351$, $p < 0.001$), and the average below the knee (BTK) score ($r^2 = 0.119$, $p = 0.006$). Presence of CLI ($r^2 = 0.154$, $p = 0.012$) and the Rutherford stage ($r^2 = 0.194$, $p = 0.003$) were associated with CML levels. We demonstrate a relationship between tAGE and the symptom profile of patients with PAD and an association between tAGE and infrapopliteal angiographic disease severity. Both tAGE and CML levels were related to the presence of CLI. These data suggest that AGE levels may reflect the severity of PAD and may be of importance in CLI.

Keywords

- ▶ peripheral arterial disease
- ▶ advanced glycation
- ▶ critical limb ischemia
- ▶ atherosclerosis

Emerging evidence implicates a central role of advanced glycation end products (AGEs) in the inflammatory and oxidative pathways leading to the development of atherosclerosis and its progression. Population studies, in cohorts with and without diabetes, have demonstrated that serum AGE levels are predictive of future adverse cardiovascular events.^{1–4} Furthermore, smaller cohort studies have shown that detection of AGEs, either by plasma quantification or skin

autofluorescence (SAF) measurement, is associated with the presence and extent of coronary artery disease (CAD).^{5,6}

In contrast, the pathophysiological role of AGEs and their utility as a biomarker for lower extremity peripheral arterial disease (PAD) remains less certain. AGE concentration as assessed by SAF appears to be related to the extent of noncoronary atherosclerosis in patients with carotid artery disease.⁷ Moreover, plasma levels of specific AGE moieties in unselected

diabetic patients and in healthy subjects are related to the presence and severity of PAD as assessed by the ankle brachial index (ABI).^{8,9} The purpose of the present study was to examine the relationship between plasma levels of AGEs and the clinical and angiographic characteristics of patients with highly symptomatic PAD undergoing invasive evaluation in the catheterization laboratory. We hypothesized that AGE levels, specifically total AGE levels (tAGE) and the specific moiety (N-carboxymethyllysine [CML]), would be related to the presence of diabetes, correlated with the ABI, and related to the presence of more extensive lower extremity atherosclerosis.

Patients and Methods

Patient Selection

Study was approved by the University of California San Diego Human Research Protections Program and all patients signed informed consent. A total of 40 consecutive patients with a de novo diagnosis of symptomatic lower extremity PAD arriving to the catheterization laboratory for peripheral angiography and/or peripheral endovascular intervention were prospectively enrolled. Data regarding comorbidities, medication use, and medical history was obtained by chart review and augmented by patient reported data. The presence of type-2 diabetes was confirmed by chart review and confirmed by the use of diabetic medications. Estimation of the glomerular filtration rate (eGFR) was made using the Cockcroft–Gault formula. Hypertension was diagnosed by chart review and confirmed by the presence of elevated systolic blood pressure (≥ 140 mm Hg) or the use of antihypertensive medication. The patient's clinical PAD profile was assessed using the Rutherford staging scale.¹⁰ The presence of critical limb ischemia (CLI) was defined as rest pain, minor tissue loss, or major tissue loss. On the morning of enrollment, all patients underwent measurement of the ABI according to previously described methods.¹¹

Serum Sample Measurements

All subjects were studied in a fasting supine state. Baseline complete blood counts and serum chemistries (including total protein) were obtained by peripheral venipuncture on the morning of enrollment. Samples for high-sensitivity C-reactive protein (hsCRP), tAGE, and CML were obtained from a 5 or 6F arterial sheath placed in the femoral artery before any contrast administration. Samples were immediately processed with extraction of the plasma and storage at -80°C until analysis. All sample analysis was performed by an experienced individual blinded to the study details. hsCRP was measured using a double antibody sandwich electrochemiluminescence immunoassay developed by Meso Scale Discovery (MSD, Gaithersburg, MD). tAGE levels were determined using a commercially available AGE ELISA Kit (Cell Biolabs Inc., San Diego, CA). This assay detects multiple glycated structures, including pentosidine, CML, N (carboxyethyl)lysine, methylglyoxal lysine dimer, glyoxal-derived lysine dimer, 3-deoxyglucosone-derived lysine dimer, and pyrraline. In addition, CML was specifically measured using an anti-CML specific monoclonal antibody based ELISA Kit (Cell Biolabs Inc.). The AGE and CML protein adduct content in the unknown samples was determined by comparison with a

standard curve that was prepared from AGE-bovine serum albumin (BSA) and CML-BSA standards, respectively. The AGE and CML concentrations were normalized for serum total protein concentration.

Angiographic Protocol and Analysis

Patients underwent peripheral digital subtraction angiography via the femoral approach. All patients underwent a static pelvic angiogram to evaluate the distal aorta and iliac vessels. The ipsilateral limb was imaged with a bolus injection and run off to the foot through the sheath while the contralateral limb was imaged through a 5F Omniflush (Angiodynamics, Latham, NY) catheter placed in the opposite external iliac artery again with a run off to the foot. The angiographic images were stored digitally online and analyzed at a later date by an experienced observer blinded to the clinical data.

The angiograms were scored by using a semiquantitative system. Stenosis severity, as defined as percentage diameter luminal stenosis, was graded by visual inspection according to the following scale, derived from a modification of the Bollinger method: 0 = no stenosis, 1 = mild (0–25%), 2 = moderate (25–50%), 3 = severe (50–99%), and 4 = occlusion (100%).¹² Grading was performed bilaterally in each limb for the following arteries: common and external iliac, common femoral, superficial femoral artery, popliteal artery, tibio-peroneal trunk, anterior tibial artery, posterior tibial artery, and peroneal artery. The most severe stenosis in each vessel in either limb was then used to derive an average score for above the knee (ATK) vessels, which included the iliac and femoropopliteal circulation and below the knee (BTK), vessels which included all the infrapopliteal vessels.

Statistical Analysis

Baseline continuous demographic and medical characteristics were compared using the Wilcoxon rank sum test. Continuous variables included age in years, weight, body mass index, ABI, systolic blood pressure, diastolic blood pressure, mean arterial pressure, pulse pressure, hemoglobin A1c, ejection fraction, blood urea nitrogen, eGFR, fasting glucose, hemoglobin A1c, hemoglobin, white blood cells, platelets, total cholesterol, low-density lipids, high-density lipids, triglycerides, hsCRP, known PAD, the average stenosis score for the ATK and the BTK and Rutherford stage. The normality of each continuous variable was evaluated using the Shapiro–Wilks statistic. For those continuous variables that deviated significantly from the assumption of normality either the variable was transformed or the central limit theorem was applied. Baseline categorical demographic and medical characteristics were compared using the Fisher exact test. Categorical variables included gender, smoking status, diabetic neuropathy, diabetes, hypertension, dyslipidemia or hyperlipidemia, CAD, prior coronary angiography, history of PCI, history of CABG, prior myocardial infarction, history of CVA or TIA, history of congestive heart failure, Rutherford stage, presence of CLI, and individual medication use. The association of the grouping variable (the presence of diabetes) on the primary (AGE) and secondary (CML) continuous (tAGE and CML) outcome variables, controlling for covariates, was

performed using multivariate regression (tAGE and CML). An initial univariate screen of covariates was performed using either the Fisher exact test or Wilcoxon rank sum test. Significant covariates ($p < 0.05$) from the univariate screen were included in the multivariate analyses.

Statistical comparisons and data management were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC) running on a Windows 7 platform. The significance level was set a priori at 0.05 (two tailed).

Results

Baseline Subject Characteristics

The baseline subject characteristics are shown in ►Table 1. Majority of the subjects were males (65%) with a mean age of 67.4 ± 8.9 years. There was a high prevalence of cardiovascular risk factors, including type-2 diabetes (65%), hypertension (92.5%), and dyslipidemia (92.5%). In addition, 28 subjects (70%) had previous-documented CAD (at least 50% diameter stenosis) by angiography and 12 (30.0%) had a previous myocardial infarction. There was a high usage of evidence-based cardiovascular medications including angiotensin-converting enzyme-I/angiotensin receptor blockers (73%), aspirin (93%), and statins (85%). A total of 17 patients underwent clinically indicated endovascular treatment (angioplasty with or without stenting) of their PAD on the day of the study.

A total of 16 (40%) patients presented with CLI, while the remaining 24 presented with a lifestyle-limiting claudication. The mean Rutherford stage was 3.4 ± 1.4 and mean ABI was 0.70 ± 0.49 . The overall study population had more severe infrapopliteal disease (BTK score 2.54 ± 1.14) as compared with ATK disease (ATK score 1.87 ± 0.76), $p = 0.003$. Visible vessel wall calcification (by fluoroscopy) of at least one major infrapopliteal vessel was present in 28 (70%) of the entire cohort and in 15 (94%) of the patients with CLI. Mean tAGE levels were 0.557 ± 0.323 $\mu\text{g/mL}$ and mean CML levels were 1.49 ± 0.412 ng/mL .

Diabetic Subgroup

Diabetics presented with a higher Rutherford stage as compared with nondiabetics (3.73 ± 1.46 vs. 2.79 ± 1.25 , $p = 0.040$). Diabetics had similar BTK scores (2.56 ± 1.20 vs. 2.50 ± 1.05 , $p = 0.889$) but lower ATK scores (1.64 ± 0.80 vs. 2.29 ± 0.45 , $p = 0.030$) as compared with nondiabetics. The majority of diabetics (20 subjects, 77%) had visible fluoroscopic calcification of the infrapopliteal vessels. Diabetics had higher tAGE levels (0.638 ± 0.327 vs. 0.408 ± 0.266 $\mu\text{g/mL}$, $p = 0.030$) and similar CML levels (1.55 ± 0.420 vs. 1.38 ± 0.387 ng/mL , $p = 0.216$) as compared with nondiabetics. In diabetic patients, there were no relationships noted between tAGE or CML levels, and fasting glucose, hemoglobinA_{1c} (HbA_{1c}) percentage, or type of diabetic medication usage (data not shown).

Univariate and Multivariate associations between tAGE Levels and Clinical Variables

The univariate associations between clinical variables and tAGE levels are shown in ►Table 2. The presence of diabetes,

diabetic neuropathy, platelet count, the presence of CLI, Rutherford stage, and BTK score are significantly associated with tAGE levels. Notably, age, fasting glucose, HbA_{1c}%, eGFR, the presence of HTN, lipid parameters, smoking, hsCRP, and the ATK score were not associated with tAGE levels. Furthermore, there were no associations noted between tAGE, CML levels, and the ABI (►Fig. 1A, B).

The significant covariates obtained from the univariable screening ($p < 0.05$) were entered into stepwise multivariable regression models (►Table 3). The separate models were evaluated, one that included CLI, one that included Rutherford stage, and one that included average (AVG) BTK score as the primary metric for PAD. There were significant relationships noted between tAGE levels and the presence of CLI (partial $r^2 = 0.195$, $p = 0.003$), Rutherford stage (partial $r^2 = 0.351$, $p < 0.001$), AVG BTK score (partial $r^2 = 0.119$, $p = 0.006$), and platelet count (partial $r^2 = 0.209$, $p < 0.001$). The graded relationship between tAGE levels and Rutherford stage is specifically demonstrated in ►Fig. 2A. Of note, when the above variables were included, the presence of diabetes was not associated with tAGE levels ($p > 0.10$) in any multivariate model.

Univariate and Multivariate associations between CML Levels and Clinical Variables

The univariate associations between CML levels and clinical variables are also shown in ►Table 2. Male gender, systolic and mean blood pressure, platelet count, the presence of CLI, Rutherford stage, and the ATK score were all associated with CML levels. The covariates obtained from the univariate screening ($p < 0.05$) were entered into stepwise multivariable regression models. In multivariate analyses, two separate models were evaluated, one that included CLI and one that included Rutherford stage as the primary metric for PAD. In the CLI model, the presence of CLI (partial $r^2 = 0.154$, $p = 0.012$) was the only variable significantly associated with CML levels. In the Rutherford stage model, male gender (partial $r^2 = 0.096$, $p = 0.040$) and Rutherford stage (partial $r^2 = 0.194$, $p < 0.003$) were significantly associated with CML levels. The relationship between Rutherford stage and CML levels is shown in ►Fig. 2B.

Discussion

There are several novel findings in the present study as follows: (1) in patients with symptomatic PAD, plasma tAGE levels are primarily related to the symptomatic presentation (Rutherford stage), presence of CLI, and angiographic severity of BTK small vessel disease; (2) CML levels are associated with the presence of CLI but not with angiographic severity of disease; (3) diabetics with PAD have higher tAGE levels than nondiabetics, however, diabetes is not an independent predictor of elevated tAGE levels; (4) tAGE levels do not correlate with the ABI in highly symptomatic patients. The following discussion frames the significance of these findings in the context of the known literature concerning AGEs and atherosclerosis.

Comparison to Published Data on AGEs and PAD

Only a limited number of studies have specifically examined the relationship between AGEs and PAD. Lapolla et al

Table 1 Baseline subject characteristics

Subject characteristics	Data
Demographic data	
Age (y)	67.4 ± 8.9
Male gender <i>n</i> (%)	26 (65.0)
Body mass index (kg/m ²)	28.2 ± 6.5
Systolic blood pressure (mm Hg)	151.2 ± 26.0
Diastolic blood pressure (mm Hg)	71.1 ± 9.8
Smoker	14 (35.0)
Diabetes	26 (65.0)
Insulin-dependent diabetes	8 (20)
Hypertension	37 (92.5)
Dyslipidemia	37 (92.5)
Coronary artery disease	28 (70.0)
Prior coronary angiography	25 (62.5)
History of percutaneous coronary intervention	20 (50)
History of coronary artery bypass	12 (30)
Prior myocardial infarction	12 (30)
History of stroke or transient ischemic attack	4 (10)
History of congestive heart failure	4 (10)
Peripheral arterial disease characteristics	
Ankle brachial index	0.70 ± 0.49
Rutherford stage	3.4 ± 1.4
Critical limb ischemia present	16 (40)
Unilateral lower extremity symptoms	18 (45)
Above the knee angiographic score	1.87 ± 0.76
Below the knee angiographic score	2.54 ± 1.14
Visible calcification of infrapopliteal vessel(s) by fluoroscopy	28 (70)
Laboratory data	
Serum creatinine (mg/dL)	1.14 ± 0.57
Estimated glomerular filtration rate (mL/min/1.73 m ²)	82.4 ± 48.2
Fasting glucose (mg/dL)	162.1 ± 91.2
Hemoglobin A _{1c} %	7.4 ± 2.5
Hemoglobin (g/dL)	12.1 ± 1.8
White blood cell count (<i>n</i> × 10 ³)	9.1 ± 5.2
Platelet count (<i>n</i> × 10 ³)	243.9 ± 87.4
Total cholesterol (mg/dL)	162.4 ± 41.5
Low-density lipoprotein cholesterol (mg/dL)	88.4 ± 38.6
High-density lipoprotein cholesterol (mg/dL)	45.8 ± 17.5
Triglycerides (mg/dL)	150.8 ± 72.7
hsCRP (mg/L), (median, interquartile range)	4.42 (2.09–12.26)
Total advanced glycation end product (μg/mL)	0.557 ± 0.323
N-carboxymethyllysine (ng/mL)	1.49 ± 0.412
Medications	
Insulin	15 (37.5)

(Continued)

Table 1 (Continued)

Subject characteristics	Data
Metformin	11 (27.5)
Sulfonylurea	5 (12.5)
TZD	0 (0)
Beta-blocker	33 (82.5)
ACE-I or ARB	31 (77.5)
Diuretic	16 (40)
Aspirin	40 (100)
Thienopyridine	32 (80)
Statin	37 (92.5)

Abbreviations: ACE-I, angiotensin-converting enzyme-I; ARB, angiotensin receptor blockers; TZD, thiazolidinediones.
Note: Data are shown as mean ± SD, or as n (% of population).

examined the serum levels of tAGEs and the specific moiety pentosidine in an unselected consecutive population of diabetics. These subjects were also screened for the presence of PAD by the ABI (defined as ABI < 0.9).⁸ The authors noted that in the subset of individuals with diabetes and PAD there was a strong inverse correlation between the ABI and tAGE and pentosidine levels. Furthermore, AGE and pentosidine levels were significantly higher in subjects with PAD as compared with healthy controls. The subjects, in general, had evidence of mild-to-moderate burden of PAD by ultrasound. Specifically, none had ATK obstructive disease and none had BTK calcifications. More recently, Takahashi et al examined pentosidine levels and a variety of cardiovascular risk factors in healthy male individuals.⁹ The authors noted that serum pentosidine levels were an independent determinant of ABI levels. Notably, as these subjects were without apparent cardiovascular disease, there was a narrow range of ABI values: mean ABI was 1.16 ± 0.07 with the lowest ABI value of 0.98.

In contrast to the above studies, there was no statistically significant association between the ABI and serum levels of tAGE or CML in the present study. Numerous differences underlie the findings between the present data and aforementioned data. We enrolled many patients which had evidence of severe infrapopliteal disease. Patients with advanced infrapopliteal atherosclerosis and diabetics with medial calcification may have poorly compressible tibial vessels decreasing the sensitivity of the ABI for detecting obstructions.^{13–15} Moreover, our study included a highly selected population of very symptomatic individuals with advanced PAD including patients with CLI. Stein et al have demonstrated that individuals with rest ischemia or ulceration can have a normal resting ABI up to 50% of the time.¹⁴ Given these limitations, the resting ABI likely provided poor stratification of the extent of atherosclerosis in the present study. Recently, using autofluorescence, de Vos et al recently demonstrated higher levels of skin deposition of AGEs in patients with PAD as compared with patients without PAD. These authors also noted a poor correlation between ABI level and AGE deposition.¹⁶

As opposed to using a noninvasive surrogate, we demonstrate, for the first time, that the angiographically determined extent of BTK atherosclerosis correlates with tAGE levels, (representative examples of this finding are shown in ►Fig. 3A, B). The epidemiology of large vessel (ATK) versus small vessel (BTK) disease has been previously described and suggests a different pathophysiological profile for each vascular distribution.^{17,18} The smaller BTK vessels, particularly in diabetics appear to be prone to atheroma development and progression. AGEs have been strongly linked to microvascular complications and may represent another key mediator of small vessel atherosclerosis.^{19–21} These data are further supported by the strong association between tAGE levels and CLI—which invariably is a disease involving the infrapopliteal vessels.

Role of AGEs in Atherosclerosis and Localization of AGEs in Peripheral Plaques

Mechanistic studies have imputed that AGEs are important mediators of vascular pathology including activation of inflammatory pathways, promotion of oxidative stress, glycation of low-density lipoprotein (LDL) (promoting oxidation of LDL), and trapping of LDL in arterial walls.²² Studies in humans, particularly in diabetics, have demonstrated the acute and subacute role of dietary AGEs in increasing measures of inflammation and inducing endothelial dysfunction.^{23,24} Furthermore, AGEs have been localized in coronary vessel samples obtained from diabetic patients directly demonstrating their presence within atheromas.²⁵ The above mentioned data coupled with emerging epidemiologic studies, suggest that AGEs may not only play a mechanistic role in CAD but also serve as biomarkers of disease activity.

A similar level of data are lacking for PAD. The results of the present study demonstrate that AGE levels correlated with the clinical presentation of PAD and the severity of BTK disease independent of the presence of diabetes, fasting glucose level, or HbA_{1c}%. These findings suggest that the contribution of AGEs to the peripheral atherosclerosis may not necessarily be limited to the diabetic state, but rather a function of the aforementioned processes of inflammation

Table 2 Univariate associations between AGE and CML levels and clinical variables

Variable	AGE		CML	
	r ²	p-Value	r ²	p-Value
Age	0.009	0.852	0.007	0.872
Male gender	0.065	0.112	0.118	0.030
Weight	0.038	0.226	0.019	0.392
Body mass index	0.016	0.433	0.032	0.273
Diabetes	0.118	0.030	0.040	0.216
HbA _{1c} %	0.096	0.051	0.027	0.311
Fasting glucose	< 0.001	0.943	0.001	0.955
Diabetic neuropathy	0.103	0.044	0.017	0.419
Log estimated GFR	0.019	0.396	0.002	0.799
Hypertension	0.026	0.324	0.003	0.727
Systolic blood pressure	0.062	0.124	0.114	0.034
Diastolic blood pressure	0.021	0.378	0.034	0.252
Mean arterial pressure	0.068	0.103	0.121	0.028
Pulse pressure	0.038	0.231	0.070	0.099
Total cholesterol	0.092	0.600	0.090	0.606
Low-density cholesterol	0.002	0.793	< 0.001	0.977
High-density cholesterol	0.040	0.252	0.010	0.569
Triglycerides	0.092	0.080	0.007	0.620
Prior myocardial infarction	< 0.001	0.978	0.008	0.859
Prior unstable angina	0.014	0.472	0.003	0.915
Smoker	0.044	0.193	0.003	0.754
History of percutaneous coronary intervention	0.010	0.544	0.051	0.163
History of coronary artery bypass grafting	0.016	0.442	0.003	0.721
History of coronary artery disease	0.090	0.060	0.090	0.060
History of stroke or transient ischemic attack	0.007	0.605	< 0.001	0.865
History of heart failure	0.071	0.095	0.056	0.141
Log white blood cell count	0.007	0.618	0.042	0.204
Log hemoglobin	0.009	0.570	0.052	0.156
Log platelet count	0.232	0.002	0.131	0.022
Log high-sensitivity C-reactive protein	0.007	0.613	< 0.001	0.955
Presence of critical limb ischemia	0.270	0.001	0.187	0.005
Log ankle brachial index	0.010	0.560	0.017	0.439
Rutherford stage	0.449	< 0.001	0.238	0.001
Above the knee angiographic score	0.017	0.100	0.110	0.037
Below the knee angiographic score	0.125	0.030	0.031	0.277

Abbreviations: GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}.

and oxidative stress—which are more common to diabetics. Halushka et al have previously attempted to localize AGEs within postmortem human vascular tissue using immuno-histochemistry staining of tissue microarrays.²⁶ The authors confirmed AGE staining in the carotid, coronary, dorsalis pedis, iliac, internal mammary, mesenteric, and pulmonary arteries.

Specific Delineation of AGE Subtypes

As AGEs represent a diverse family of molecules, the precise moieties which are key mediators of atherosclerosis remain uncertain. In the present study, the tAGE levels represented a nonspecific global measure of multiple glycated products therefore it was not possible to decipher if the findings are related to the presence of a specific AGE. Marked elevation of

Fig. 1 (A) Relationship between plasma total AGE (tAGE) levels and the ankle brachial index (ABI). tAGE levels do not correlate with the ABI in highly symptomatic patients ($r = 0.074$, $p = 0.659$). (B) Relationship between plasma N-carboxymethyllysine (CML) levels and the ABI. CML levels do not correlate with the ABI in highly symptomatic patients ($r = 0.055$, $p = 0.741$).

Fig. 2 (A) Graded relationship between total AGE (tAGE) levels and the Rutherford stage. tAGE levels are related to the severity of peripheral arterial disease (PAD) presentation in a graded fashion ($p < 0.001$). (B) Relationship between N-carboxymethyllysine (CML) levels and the Rutherford stage. CML levels are related to the severity of PAD presentation but not in a graded fashion as was noted for tAGE levels ($p = 0.003$ for the overall relationship).

Table 3 Independent predictors of tAGE and CML levels by multivariate analysis

Variable ^a	Parameter estimate	r ² (partial)	r ² (model)	p-Value
tAGE				
Model 1				
Critical limb ischemia	0.300	0.195	0.313	0.003
Model 2				
Rutherford stage	0.140	0.351	0.469	< 0.001
Model 3				
Average below the knee score	0.104	0.119	0.446	0.006
Platelet count	0.002	0.209	0.446	< 0.001
CML				
Model 1				
Rutherford stage	0.132	0.194	0.330	0.003
Male gender	−0.261	0.096	0.330	0.035
Model 2				
Critical limb ischemia	0.339	0.154	0.193	0.012

Abbreviations: CML, N-carboxymethyllysine; tAGE, total advanced glycation end product.
^aEach model included the presence of diabetes as the grouping variable.

Fig. 3 (A) Representative angiogram from the study demonstrating minimal below the knee (BTK) atherosclerosis in a diabetic patient with lifestyle-limiting claudication. The patient had focal bilateral superficial femoral artery disease (above knee anatomy not shown) (Rutherford stage 2.0, BTK score 0.25, total AGE [tAGE] 0.301 $\mu\text{g}/\text{mL}$, N-carboxymethyllysine [CML] 1.23 ng/mL , HbA_{1C}% 13.1). (B) Representative angiogram from the study demonstrating extensive BTK atherosclerosis in a diabetic patient with a nonhealing foot ulcer. Note the higher tAGE levels, but similar CML levels. Also, note that the tAGE levels were independent of the degree of glycemic control (Rutherford stage 6.0, BTK score 3.75, tAGE 0.796, CML 1.20, HbA_{1C}% 6.7). Arrows demonstrate the numerous areas of atherosclerosis BTK.

CML, which can be produced either by glycooxidation or lipoxidation pathways, demonstrated an association with the presence of CLI, but this marker was less robustly related to the severity of BTK atherosclerosis. These findings suggest that a systematic approach will be needed to delineate the specific AGEs which can be considered as tools for the detection and assessment of PAD presence and severity.

AGEs and Foot Ulcers

In the present study, Rutherford 5 and 6 stage patients (those with nonhealing ulcers) had the highest plasma levels of AGEs. This finding was independent of hsCRP levels suggesting an association not related to an acute phase inflammatory response. There is a small, but growing body of evidence which implicates AGEs directly in the impairment of chronic wound healing. Animal studies have demonstrated that exogenous dietary AGE administration increases wound

AGE levels, and elevated-AGE levels impaired neutrophil and macrophage responses.^{27,28} The interaction between AGE and receptor for advanced glycation endproducts (RAGE) has been linked to an abnormal inflammatory response in murine diabetic wounds and binding of AGE by soluble RAGE appears to enhance wound healing.²⁹ The impact of AGEs on wound healing appear to be related to multiple processes including altering the inflammatory cascade, fibroblast function, cellular apoptosis, and oxidative pathways. The pathological role of AGEs in individuals with foot ulcers remains to be better elucidated; as does their role in contributing to the often coexistent small vessel atherosclerosis seen in these patients.

Study Limitations

The present study is limited in sample size and is therefore the data are not definitive but rather hypothesis generating in

nature. Larger prospective studies will be needed to confirm our findings. As we enrolled highly symptomatic patients arriving for angiographic analysis, the generalizability to the overall PAD population is limited. In addition, due to the small sample size and the cross-sectional design we did not explore the role of tAGE and CML levels on the clinical outcomes. In addition, the patients were all on aggressive medical therapy with high rate of aspirin, statin, and diabetic medication use which may have impacted the levels of AGEs. In particular, 11 patients were taking metformin which may have “anti-AGE” effects.³⁰ As noted above, diabetic complications (including nephropathy, retinopathy, and neuropathy) were not directly assessed—limiting our analyses in the diabetic subgroup. Finally, the role of dietary AGEs cannot be overlooked. Multiple studies have highlighted the acute impact of various foodstuffs in elevating blood AGE levels.^{31,32} Although all of our subjects were fasting, the influence of subacute and/or chronic dietary AGE ingestion could have influenced the findings and represents a notable limitation. Finally, there remains uncertainty on not only which AGE moiety is clinically relevant but also the best assay method to measure these entities. This practical limitation makes it difficult to compare results across studies—include with our own.

Conclusions

We demonstrate a relationship between tAGE levels and the symptom profile of patients with PAD. We also demonstrate an association between tAGE and BTK angiographic severity of atherosclerosis. Both tAGE and CML levels were related to the presence of CLI and Rutherford stage—however, this relationship was more robust for tAGE levels. These data suggest that AGE levels may reflect the severity of PAD and may be of particular importance in patients with CLI. Further studies exploring the above associations are warranted.

Conflict of Interest

No conflict of interest to report.

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