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Serum carboxymethyl-lysine, an advanced glycation end product, is associated with arterial stiffness in older adults

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Abstract

Objective—To examine the relationship of serum carboxymethyl-lysine (CML), an advanced glycation end product (AGE), with pulse pressure (PP), aortic pulse wave velocity (aPWV), and hypertension in older adults.

Background—AGEs are bioactive molecules that accumulate in tissues with aging and can both cross-link collagen and induce inflammation in model systems. The relationship of AGEs with arterial stiffness and hypertension has not been well characterized in community-dwelling older adults.

Methods—We measured serum CML and blood pressure in 3044 adults, aged 70–79 y, who participated in the Health, Aging and Body Composition Study, a population-based study of aging in Pittsburgh, Pennsylvania, and Memphis, Tennessee. aPWV was measured in 2468 participants.

Results—Participants in the highest tertile of serum CML had higher PP (highest tertile: beta = 2.85, SE = 0.82, *P* = 0.0005; middle tertile: beta = 0.60, SE = 0.80, *P* = 0.45), and higher aPWV (highest tertile: beta = 51.4, SE = 20.1, *P* = 0.01; middle tertile: beta = 3.2, SE = 19.8, *P* = 0.87)

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Conflicts of interest:
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compared with those in the lowest tertile in multivariable linear regression models adjusting for age, sex, race, education, BMI, smoking, alcohol use, total cholesterol, HDL cholesterol, diabetes, cardiovascular disease, and chronic kidney disease. Participants in the highest and middle tertiles of serum CML had higher odds of hypertension (Odds Ratio [O.R.] 1.32, 95% Confidence Interval [C.I.] 1.06, 1.60, $P = 0.005$; O.R. 1.27, 95% C.I. 1.05, 1.53, $P = 0.01$, respectively) compared with the lowest tertile in a multivariable logistic regression model adjusting for the same covariates.

Conclusions—Elevated serum CML was associated with arterial stiffness, as reflected by higher PP and aPWV, in older, community-dwelling adults.

Keywords

advanced glycation end products; aortic pulse wave velocity; arterial stiffness; carboxymethyl-lysine; hypertension; pulse pressure

Introduction

Arterial stiffness increases with age [1] and is associated with a greater risk of hypertension, coronary artery disease, stroke, and cardiovascular mortality [2–8]. In addition, loss of elasticity of the large arteries results in the transmission of excessive pulsatile energy into the microcirculation, where it can cause damage to the brain and kidneys [9,10]. Age-related arterial stiffness is associated with increases in collagen and degeneration and fragmentation of elastin [11]. Aortic pulse wave velocity (aPWV), the gold standard method for the measurement of aortic stiffness, is an important indicator of early vascular damage and cardiovascular risk [12].

Factors that are associated with arterial stiffness are not well understood but include inflammation and oxidative stress [13], serum lipids [14], apolipoprotein B [15], and advanced glycation end products (AGEs) [16]. AGEs are bioactive molecules that are generated in the body through abnormal glucose metabolism, increased lipid peroxidation, and inflammation [16]. AGEs are formed from the non-enzymatic glycation of proteins, lipids, and nucleic acids. In the Maillard reaction, reducing sugars react with lysyl residues of proteins to form intermediate Amadori products, which then undergo further complex reactions to give rise to AGEs such as carboxymethyl-lysine (CML), glucosepane, hydroimidazolone, and pentosidine [17]. With aging, there is decreased turnover of elastin and collagen in the large arteries and accumulation of AGEs within arteries [17,18]. Diastolic dysfunction in older men has been associated with elevated plasma CML concentrations [19]. In diabetic rats, AGEs increase vascular stiffness by inducing collagen cross-linking in the vessel wall [17, 20]. CML may potentiate breakdown of elastin by binding active redox metals and inducing lipid peroxidation [21].

The relationship between circulating CML, arterial stiffness, and hypertension has not been well characterized in community-dwelling older adults. We hypothesized that older adults with elevated serum CML would have greater risk of arterial stiffness and hypertension. To address this hypothesis, we examined the relationship between serum CML and pulse pressure (PP), aPWV, and hypertension, respectively, in a population-based cohort of older adults.

Materials and Methods

Study Population

The subjects of this study were participants in the Health, Aging and Body Composition (Health ABC) Study, a community-based prospective study of the impact of changes in weight and body composition on age-related physiological and functional changes. Participants (1491 men and 1584 women) aged 70–79 y were recruited between March 1997 and July 1998 from two sites in Pittsburgh, PA and Memphis, TN. Participants were drawn from a simple random sample of Medicare beneficiaries residing in zip codes from the metropolitan areas surrounding Pittsburgh and Memphis. Medicare covers health insurance for the vast majority of older adults and provided >96% coverage of adults ≥65 years in 2000 [22]. Participants were eligible if they reported no difficulty climbing ten steps, walking one-quarter of a mile, or performing basic activities of daily living, had no life-threatening illnesses, were not participating in another study involving alterations to diet, medications, or exercise habits, and intended to remain within the area for three or more years.

Prevalent medical conditions were evaluated by questionnaire and confirmed by use of specific medications or procedures. Hypertension was defined as self-reported physician diagnosis of hypertension confirmed by use of an anti-hypertensive medication or an elevated measured blood pressure (>=140/90), regardless of the history or medication use. Prevalent cardiovascular disease included myocardial infarction, angina, stroke, or transient cerebral ischemia or any revascularization procedure, including endarterectomy or angioplasty. Body mass index (BMI) was expressed as weight in kilograms divided by height in meters squared. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation of Levey and colleagues [23]. All participants gave written informed consent. The Institutional Review Boards of the clinical sites, the Data Coordinating Center of the University of California, San Francisco, and the Johns Hopkins School of Medicine approved the protocol for the study.

Measurement of aPWV

Measurements of aPWV in this study have been described in detail elsewhere [4]. Briefly, aPWV was measured from simultaneous Doppler flow signals obtained from the right carotid and right femoral arteries with nondirectional transcutaneous Doppler flow probes (model 810A, 9.0- to 10.0-MHz probes, Parks Medical Electronics, Inc). Three separate runs were recorded for each participant, and all usable runs were averaged. The National Institute on Aging, Laboratory of Cardiovascular Science, Gerontology Research Center (Baltimore, MD) trained and certified all study personnel before data collection, read the waveforms, and evaluated data quality. Results from all acceptable runs were averaged for the final aPWV measurement used in the analyses. Replicate measurements of aPWV in 14 subjects showed intraclass correlations of 0.88 between sonographers and 0.84 between readers.

The present analysis of aPWV is based upon aPWV measurements at enrollment from 2468 participants. There were 354 participants with missing aPWV data because of equipment problems, 233 participants with unusable waveforms, and 20 participants who had aPWV

measurements but did not have a corresponding serum sample available at the enrollment visit. Those with missing aPWV values were more likely to be black (21.5% versus 17.1%, $P = 0.002$), had lower systolic blood pressure (133 versus 136 mg Hg, $P = 0.004$), but similar serum CML concentrations (815 versus 798 ng/mL, $P = 0.30$). There were no significant differences between those with and without aPWV measurements by age, body mass index, pulse pressure, prevalence of hypertension or other chronic diseases.

Laboratory measurements

Serum levels of C-reactive protein (CRP) and interleukin-6 (IL-6) were measured at enrollment using ELISA (Calbiochem, San Diego, CA, and Quantikine, R & D Systems, Minneapolis, MN, respectively). The CRP assay was standardized according to the World Health Organization First International Reference Standard, with a sensitivity of 0.08 μg/mL. The lower limit of detection for IL-6 was 0.10 pg/mL. CML was measured at enrollment using an enzyme-linked immunosorbent assay (ELISA) (AGE-CML ELISA; Microcoat, Penzberg, Germany) [24]. This assay has been validated, is specific, and exhibits no cross-reactivity with other compounds [24,25]. Inter-assay coefficients of variation for IL-6, CRP, and CML were 15%, 5%, and 10%, respectively. Hemoglobin A_{1c} was measured using high performance liquid chromatography (Biorad Variant HPLC). Total cholesterol, HDL cholesterol, and triglycerides were measured using a Vitro 950 analyzer (Johnson & Johnson). LDL cholesterol levels were calculated using the Friedewald equation [26].

Statistical Analysis

Continuous variables were compared between groups using rank sum tests. Continuous covariates with a skewed distribution were log transformed to normalize the distribution. Categorical variables were compared between groups using chi-square tests. Primary predictor variable was CML. Outcomes examined were pulse pressure (PP), aPWV, systolic and diastolic blood pressure (all continuous) and hypertension (yes/no). Multivariable linear and logistic regression models were used to examine the cross-sectional relationship between serum CML and PP, aPWV, systolic blood pressure, diastolic blood pressure, and hypertension. Conventional risk factors for arterial stiffness and hypertension were included as covariates in the multivariable models. Chronic kidney disease and diabetes were included in final multivariable models because of their strong association with serum CML concentrations. Significance of statistical associations was evaluated at an alpha-level of 0.05. All analyses were conducted using SAS 9.1 (SAS Institute, Cary, NC).

Results

The characteristics of the study participants by tertiles of serum CML are shown in Table 1. Participants with higher serum CML were older, less likely to be white or female, more likely to be never smokers, with lower alcohol consumption, lower BMI, lower C-reactive protein and IL-6, lower triglycerides, and with higher hemoglobin A_{1c}, lower total cholesterol, higher HDL cholesterol, lower triglycerides, lower eGFR, higher systolic blood pressure, higher PP, and higher aPWV than those with lower serum CML. The prevalence of hypertension, cardiovascular disease, coronary heart disease, diabetes, and chronic kidney disease were significantly higher among participants with higher compared with lower

serum CML. There were no significant differences in education, LDL cholesterol, or diastolic blood pressure between participants across tertiles of serum CML.

Participants in the highest tertile of serum CML had significantly higher PP and aPWV, respectively, compared with those in lowest tertile of CML in multivariable linear regression models adjusting for age, sex, race, education, and BMI (model 1), additionally for smoking, alcohol use, total cholesterol, and HDL cholesterol (model 2), and finally with addition of diabetes and chronic kidney disease (model 3) (Table 2). Participants in the highest tertile of serum CML had significantly higher odds of hypertension in logistic regression models adjusting for the same variables as in the above three models.

Since use of anti-hypertensive medications could potentially affect the relationship of serum CML with pulse pressure and aPWV, we examined multivariable models that were stratified by hypertension medication use. Nine subjects had missing data for hypertension medication use. For pulse pressure, among 1662 participants taking hypertension medications, those in the highest tertile (beta 2.80, SE 1.18, $P = 0.02$) and middle tertile (beta −0.69, SE 1.18, $P = 0.55$) were compared with those in the lowest tertile in multivariable linear regression models adjusting for age, sex, race, education, BMI, smoking, alcohol use, total and HDL cholesterol, diabetes, and chronic kidney disease. For pulse pressure, among 1373 participants not taking hypertension medications, those in the highest tertile (beta 1.97, SE 1.11, $P = 0.07$) and middle tertile (beta 1.46, SE 1.06, $P = 0.16$) were compared with those in the lowest tertile in multivariable linear regression models adjusting for the same variables as the model above. For aPWV, among 1284 participants taking hypertension medications, those in the highest tertile (beta 63.4, SE 29.0, $P = 0.02$) and middle tertile (beta 0.86, SE 29.1, $P = 0.97$) were compared with those in the lowest tertile in multivariable linear regression models adjusting for the same variables as the model above. For aPWV, among 1058 participants not taking hypertension medications, those in the highest tertile (beta 14.5, SE 27.7, $P = 0.60$) and middle tertile (beta −1.34, SE 26.5, $P = 0.93$) were compared with those in the lowest tertile in multivariable linear regression models adjusting for the same variables as the model above.

As statistical power is decreased by stratification, we alternatively analyzed the relationship of serum CML with pulse pressure and aPWV in multivariable models that adjusted for use of hypertension medications. For pulse pressure, those in the highest tertile (beta 2.40, SE 0.81, $P = 0.003$) and middle tertile (beta 0.30, SE 0.79, $P = 0.70$) were compared with those in the lowest tertile in multivariable linear regression models adjusting for hypertension medication use and the same variables as model 3 above. For aPWV, those in the highest tertile (beta 42.9, SE 20.1, $P = 0.03$) and middle tertile (beta −1.34, SE 19.7, $P = 0.94$) were compared with those in the lowest tertile in multivariable linear regression models adjusting for hypertension medication use and the same variables as the models above.

We also examined the relationships of serum CML with systolic and diastolic blood pressure, respectively. Participants in the highest tertile but not the middle tertile of serum CML had higher systolic blood pressure compared to those in the lowest tertile of CML (beta = 2.27, SE = 0.96, $P = 0.02$; beta = 0.59, SE = 0.95, $P = 0.52$, respectively) in a multivariable linear regression model adjusting for the same variables as the models above.

There was no relationship of the highest tertile or middle tertile of serum CML with diastolic blood pressure when compared with the lowest tertile in a similar multivariable linear regression model (beta = −0.59, SE = 0.52, *P* = 0.27; beta = −0.005, SE = 0.52, *P* = 0.99, respectively).

Among 1662 participants taking hypertension medications, for systolic blood pressure, those in the highest tertile (beta 2.34, SE 1.38, *P* = 0.09) and middle tertile (beta −0.54, SE 1.37, *P* = 0.69) were compared with those in the lowest tertile in multivariable linear regression models adjusting for the same variables as the model above. Among 1373 participants not taking hypertension medications, for systolic blood pressure, those in the highest tertile (beta 1.66, SE 0.96, *P* = 0.08) and middle tertile (beta 0.21, SE 0.94, *P* = 0.83) were compared with those in the lowest tertile in multivariable linear regression models adjusting for the same variables as the model above. There were no significant relationships between tertiles of CML and diastolic blood pressure either in multivariable linear regression analyses stratified by hypertension or adjusting for hypertension medication use adjusting for same variables above (data not shown).

Prevalent cardiovascular disease was significantly associated with tertiles of CML. In order to explore this association further, we ran additional multivariable models to determine whether tertiles of CML were independently associated with cardiovascular disease. The highest tertile and middle tertile of CML was not associated with cardiovascular disease (O.R. 1.13, 95% C.I. 0.91, 1.41, *P* = 0.28; O.R. 1.15, 95% C.I. 0.93, 1.43, *P* = 0.20, respectively) when compared with the lowest tertile in a multivariable logistic regression model adjusting for age, sex, race, BMI, smoking, mean arterial pressure, fasting plasma glucose, total cholesterol, HDL cholesterol, diabetes, and chronic kidney disease.

Discussion

The present study, based upon a large, population-based sample, suggests that older adults with elevated CML have greater arterial stiffness, as indicated by PP and aPWV, and higher odds of hypertension. AGE-induced alterations in the vasculature may contribute to greater arterial stiffness [11,16]. AGEs such as CML and glucosepane can alter the extracellular matrix by cross-linking type-IV collagen and inhibiting the association of these molecules into a normal complex network-like structure [27,28] and cross-linking with laminin to inhibit polymer self-assembly [29,30]. Other biological mechanisms by which AGEs could contribute to alterations in the vasculature include potentiation of endothelial dysfunction through impairment of nitric oxide production [31], increased oxidation of low-density lipoprotein [32], and increased production of reactive oxygen species [33]. The present study is consistent with a previous observation in which elevated CML was associated with pulse pressure in 543 adults with type 1 diabetes [34]. The findings from the present study are also consistent with the association of elevated circulating CML with higher aPWV in nearly 500 adults, aged 26–93 y, in the Baltimore Longitudinal Study of Aging [35]. This study extends the association between serum carboxymethyl-lysine and arterial stiffness in a population of exclusively septuagenarians. The relationship between elevated CML and arterial stiffness was strongest in those who were taking anti-hypertensive medications. The reasons for this association are not clear and should be corroborated in future studies. The

present study shows a cross-sectional association between AGEs and arterial stiffness, therefore a causal relationship cannot necessarily be inferred.

Elevated circulating AGEs are thought to increase systemic inflammation through binding with RAGE [36]. AGE-RAGE binding may increase the transcription of inflammatory factors such as IL-6, tumor necrosis factor-α, adhesion molecules, and other pro-inflammatory cytokines via nuclear factor kappa B [37]. However, the present study does not provide evidence that systemic inflammation is associated with higher AGEs, as higher serum CML concentrations were not associated with higher serum concentrations of either CRP or IL-6. Higher serum CML concentrations were also associated with lower BMI. Our previous studies show a strong and consistent association between high serum CML concentrations and lower BMI and fat mass [38]. Since obesity is associated with both elevated IL-6 and CRP levels [39], one possibility is that any effects of elevated circulating AGEs upon systemic inflammation are small relative to the effects of obesity and increased fat mass upon circulating IL-6 and CRP.

The pool of AGEs in the circulation is thought to derive endogenously from oxidative stress, increased lipid peroxidation, and abnormal glucose metabolism, and exogenously from dietary sources of AGEs and from smoking [16]. In the present study, higher serum CML concentrations were associated with higher hemoglobin A_{1c} and prevalent diabetes. The relationship between serum AGEs and hemoglobin A_{1c} has been inconsistent in previous studies [40–45] possibly due to limited sample sizes and differences in study populations. In the present study, the proportion of participants who never smoked was significantly higher in the highest tertile of serum CML. We have been unable to corroborate a previous study in which circulating AGEs were higher in smokers than non-smokers [46].

When foods are heated to high temperatures, the characteristic “browning” of foods is accompanied by an increase in AGE content of the food [47]. Whether diet makes a substantial contribution to circulating AGEs and has any harmful effect upon human health remains highly controversial. Observational studies have provided conflicting results. One study of sixty-nine subjects showed a highly significant correlation of $r = 0.46$ between dietary AGEs, as assessed by 3-d food records, and serum CML concentrations [48]. In a study of 261 adults, no relationship was found between either serum CML or urinary CML, respectively, with a diet high in AGEs, as assessed by six separate 24-h dietary recalls [49]. The differences in results between these two dietary studies, and between the present study and previous studies of circulating CML concentrations is unlikely to be due to differences in laboratory assay, as circulating CML was measured by ELISA using the same monoclonal antibody [48,49]. Intervention trials of dietary AGE restriction have not shown a clear impact upon health, and most of the trials to date have been of poor methodological quality [50].

In conclusion, elevated circulating CML was associated with arterial stiffness and hypertension. Whether reduction of circulating AGEs will have an effect upon existing arterial stiffness or prevent the development of arterial stiffness is not known.

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Table 1

Characteristics of 3044 participants, 70–79 years, in the Health, Aging, and Body Composition Study by tertiles of serum carboxymethyl-lysine (CML) at baseline

Characteristic[1]		**Serum carboxymethyl-lysine (ng/mL)**			***p*[2]**
		<628 n = 1014	**628–817 n = 1015**	**>817 n = 1015**	
Age (y)		73.4 (2.8)	73.6 (2.8)	73.7 (2.9)	0.02
Race, white (%)		61.0	61.7	52.6	<0.0001
Sex, women (%)		53.6	54.0	46.8	0.001
Smoking (%)	Never	40.6	43.4	47.2	0.0003
	Past	46.1	46.8	45.0	
	Current	13.3	9.8	7.8	
Alcohol consumption (drinks)	None	44.9	50.2	55.9	<0.0001
	<1/mo	21.8	19.7	20.9	
	1–7/wk	23.7	22.7	17.9	
	>1/day	9.6	7.4	5.3	
Education, years (%)	>8	24.1	25.0	26.5	0.18
	8 to 12	34.3	34.1	29.7	
	>12	41.6	40.9	43.8	
Body mass index (kg/m^2)		28.0 (4.9)	27.1 (4.8)	27.1 (4.7)	<0.0001
Log C-reactive protein (μg/mL)		0.69 (0.82)	0.62 (0.86)	0.61 (0.87)	0.01
Log interleukin-6 (pg/mL)		0.73 (0.63)	0.58 (0.63)	0.65 (0.64)	<0.0001
Hemoglobin A_{1c} (%)		6.15 (0.83)	6.27 (0.98)	6.68 (1.41)	<0.0001
Total cholesterol (mg/dL)		202 (39)	206 (38)	201 (39)	0.006
HDL cholesterol (mg/dL)		52 (16)	55 (17)	54 (17)	0.0002
LDL cholesterol (mg/dL)		121 (34)	123 (34)	121 (35)	0.14
Triglycerides (mg/dL)		143 (89)	137 (75)	135 (84)	0.003
Estimated GFR (mL/min per 1.73 m^2)		62.4 (15.4)	59.6 (15.5)	56.6 (16.8)	<0.0001
Taking anti-hypertensive medication(s) (%)		49.1	54.2	61.0	<0.0001
Systolic blood pressure (mm Hg)		134 (20)	135 (21)	137 (22)	0.02

Characteristic[1]	Serum carboxymethyl-lysine (ng/mL)			p[2]
	<628 n = 1014	628–817 n = 1015	>817 n = 1015	
Diastolic blood pressure (mm Hg)	71 (11)	71 (12)	71 (12)	0.87
Pulse pressure (mm Hg)	63.1 (17.3)	64.0 (18.4)	66.2 (18.0)	0.0003
Aortic pulse wave velocity (cm/s)	885 (368)	881 (378)	943 (431)	0.02
Hypertension (%)	56.7	61.5	64.6	0.001
Cardiovascular disease (%)	22.4	24.6	27.1	0.05
Coronary heart disease (%)	17.7	18.9	22.0	0.05
Diabetes (%)	1.5	1.7	4.9	<0.0001
Chronic kidney disease (%)	46.1	51.2	58.5	<0.0001

[1] Means (SD) for continuous variables, percentages for categorical variables.

[2] Kruskal-Wallis tests for continuous variables, chi-square tests for categorical variables.

Table 2

Multivariable regression models of the relationship of serum CML with blood pressure, pulse pressure, aortic pulse wave velocity, and hypertension in 3044 adults, 70–79 y, in the Health, Aging and Body Composition Study. Betas or odds ratios comparing highest and middle with the lowest tertile of CML[1]

Outcomes[2]	Tertiles[3] of CML	Model 1 Adjusted for age, sex, race, education, BMI			Model 2 Adjusted for age, sex, race, education, BMI, smoking, alcohol use, total cholesterol, HDL cholesterol			Model 3 Adjusted for age, sex, race, education, BMI, smoking, alcohol use, total cholesterol, HDL cholesterol, diabetes, cardiovascular disease, and chronic kidney disease		
		Beta	**SE**	***P***	**Beta**	**SE**	***P***	**Beta**	**SE**	***P***
Pulse pressure	Middle	0.83	0.79	0.29	0.69	0.80	0.39	0.60	0.80	0.45
	Highest	3.15	0.79	<0.0001	3.12	0.81	0.0001	2.85	0.82	0.0005
Aortic pulse wave velocity	Middle	0.7	19.4	0.97	6.3	19.8	0.75	3.2	19.8	0.87
	Highest	54.7	19.5	0.005	58.6	20.0	0.003	51.4	20.1	0.01
		OR	**95% CI**	***P***	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Hypertension	Middle	1.30	1.08, 1.56	0.005	1.30	1.08, 1.57	0.006	1.27	1.05, 1.53	0.01
	Highest	1.43	1.19, 1.73	0.0001	1.39	1.15, 1.68	0.0008	1.32	1.06, 1.60	0.005

[1] Multivariable logistic regression models for categorical variable hypertension; multivariable linear regression models for continuous variables pulse pressure and aortic pulse wave velocity.

[2] Analyses for pulse pressure and hypertension were conducted for all 3044 participants. Analyses for aPWV were conducted for the 2468 participants who had aPWV measurements available.

[3] The lowest tertile is the reference group for all models.