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Advanced Glycation/Glycoxidation Endproduct Carboxymethyl-Lysine and Incidence of Coronary Heart Disease and Stroke in Older Adults

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Abstract

Background—Advanced glycation/glycoxidation endproducts (AGEs) accumulate in settings of increased oxidative stress – such as diabetes, chronic kidney disease and aging – where they promote vascular stiffness and atherogenesis, but the prospective association between AGEs and cardiovascular events in elders has not been previously examined.

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Methods—To test the hypothesis that circulating levels of N^{ε}-carboxymethyl-lysine (CML), a major AGE, increase the risk of incident coronary heart disease and stroke in older adults, we measured serum CML by immunoassay in 2,111 individuals free of prevalent cardiovascular disease participating in a population-based study of U.S. adults ages 65 and older.

Results—During median follow-up of 9.1 years, 625 cardiovascular events occurred. CML was positively associated with incident cardiovascular events after adjustment for age, sex, race, systolic blood pressure, anti-hypertensive treatment, diabetes, smoking status, triglycerides, albumin, and self-reported health status (hazard ratio [HR] per SD [0.99 pmol/l] increase = 1.11, 95% confidence interval [CI]=1.03–1.19). This association was not materially attenuated by additional adjustment for C-reactive protein, estimated glomerular filtration rate (eGFR), and urine albumin/creatinine ratio. Findings were similar for the component endpoints of coronary heart disease and stroke.

Conclusions—In this large older cohort, CML was associated with an increased risk of cardiovascular events independent of a wide array of potential confounders and mediators. Although the moderate association limits CML's value for risk prediction, these community-based findings provide support for clinical trials to test AGE-lowering therapies for cardiovascular prevention in this population.

Keywords

Advanced glycation endproducts; Carboxymethyl-lysine; Aging; Older Adults; Coronary Heart Disease; Stroke

Introduction

Advanced glycation/glycoxidation endproducts (AGEs) are a diverse class of highly bioactive compounds produced when carbonyl groups on sugars react with amino groups on protein, lipid, or nucleic-acid targets.[1] While hyperglycemia can drive the initial reactions on the path to AGE formation – with glycation of hemoglobin being a prime example – subsequent oxidative reactions play a predominant role in the genesis of these molecules.[2] Apart from glycoxidation, lipid peroxidation is also involved, leading to the formation of related advanced lipoxidation endproducts (ALEs).[2] Accordingly, AGE (and ALE) levels are elevated not only in diabetes, but also in other settings characterized by increased oxidative stress, such as chronic kidney disease (CKD), advancing age, and presence of other cardiovascular risk factors.[3] Another major source of AGEs and ALEs is the diet, as such adducts are generated abundantly during cooking of foodstuffs to high temperatures.[2]

AGEs (and ALEs) have distinctive effects on the vasculature, modifying collagen and other proteins in the arterial wall to increase vascular stiffness, an important risk factor for cardiovascular disease (CVD).[4] AGEs also modify lipids and proteins in LDL, fostering LDL trapping in the subendothelial compartment.[4] Moreover, AGEs bind to an immunoglobulin superfamily receptor, known as the receptor for AGE (RAGE), which activates nuclear factor kappa B (NFkB), among other pro-oxidant and pro-inflammatory pathways.[5] NFkB is a principal orchestrator of the inflammatory response, and RAGE

expression in endothelial and vascular smooth muscle cells, as well as leukocytes, promotes atherogenesis.[5]

Despite experimental evidence implicating AGEs in vascular disease,[3, 4] and the prognostic value demonstrated for glycated hemoglobin in longitudinal cohort studies,[6] epidemiological data on the relationship between glycoxidative species and clinical CVD outcomes remain sparse. A prospective study of middle-aged adults employed a polyclonal immunoassay to measure circulating AGEs, and found these to be associated with increased risks of cardiovascular and coronary mortality in women, but not men, with[7] and without[8] diabetes. With the development of an immunoassay for N^{ε}-carboxymethyl-lysine (CML), a dominant AGE and ALE in plasma and tissue proteins,[9–11] circulating levels of CML have been associated with all-cause and cardiovascular mortality in moderate-sized population-based cohorts of generally healthy elders[12] and disabled older women.[13] The same immunoassay was used to measure serum CML, in conjunction with other AGEs, in a modest-sized cohort with CKD, but no association was detected with incident CVD.[14] By contrast, different AGEs measured by mass spectrometry/liquid chromatography, including CML, were found to be positively associated with incident CVD in patients with type 1 diabetes.[15] The extent to which circulating AGEs influence the risk of new-onset coronary heart disease (CHD) and stroke in older adults, however, has to our knowledge not been previously examined. We undertook to address this question is a prospective cohort study of community-dwelling older men and women.

Methods

Study Population

The Cardiovascular Health Study (CHS) is a population-based investigation of determinants of CVD risk among adults aged 65 and older recruited from Medicare eligibility lists in four U.S. field centers.[16] An original cohort of 5,201 subjects was recruited in 1989–90, followed in 1992–93 by a supplementary African-American cohort of 687 individuals. As described previously, participants underwent standardized health evaluations at site clinics at the time of initial enrollment and at follow-up visits.[16, 17] The study was conducted in accordance to the Declaration of Helsinki, and all participants provided informed consent.

Of the 5,888 initially recruited subjects, 4,412 out of 4,708 surviving individuals returned for the 1996–97 examination. Among these participants, 2,732 were free of prevalent CVD (CHD, heart failure, atrial fibrillation, stroke, transient ischemic attack, and peripheral arterial disease), as documented by adjudication since the baseline evaluation.[17] Exclusion of participants without available serum (n=565) or imputable covariate data (n=56) during a previous multiple imputation procedure[18] left 2,111 subjects eligible for the present analyses.

Cardiovascular Events

Surveillance for new-onset cardiovascular events entailed semiannual telephone contacts and/or clinical examinations.[19] Medical records were reviewed for potential incident events and all deaths, and events adjudicated by CHS committees according to standardized

criteria. Follow-up extending through June 2009 was >97% complete.[16, 19, 20] The primary endpoint was a composite of CHD (nonfatal myocardial infarction and fatal coronary events) and nonfatal/fatal stroke, as defined previously.[16, 17, 19]

Measurement of CML

CML measurement was performed in 2011 with an immunoassay (AGE-CML ELISA, Microcoat, Penzberg, Germany) in serum specimens stored at −70°C since collection in 1996–97. CML is a highly stable chemical species, with mean levels reported to be comparable in plasma samples frozen for 10 years and freshly drawn specimens.[21] This immunoassay has similar affinity for protein-bound, peptide-bound, and free CML.[22] The minimal detectable level of the assay is 0.02 pmol/l. Intra- and inter-assay analytical coefficients of variation were <5%.

Covariates

Diabetes was defined by fasting glucose ≥7.0 mmol/l or use of glucose-lowering therapy. Measures of body size were determined in standardized fashion.[23]

Laboratory measurements were obtained on fasting blood samples as detailed previously.[24] Homeostasis model assessment of insulin resistance (HOMA2-IR) was calculated using a standard approach,[25] as was estimated glomerular filtration rate (eGFR) based on cystatin C.[26]

Statistical Analysis

Positively skewed variables were logarithmically transformed. Differences in CML concentrations by levels of baseline covariates were assessed with Student's t test. Cross-sectional associations of CML were assessed with Spearman correlation coefficients and linear regression. The shape of the association between CML and new-onset CHD or stroke was examined with a Cox proportional hazards model adjusted for potential confounders, using a penalized cubic spline. Presence of non-linearity was tested with a partial likelihood ratio test. Testing of the proportional hazards assumption by Schoenfeld's goodness-of-fit procedures showed no material violation. Missing values for covariates at the 1996–97 examination (n=83) were replaced by values carried over from prior examinations, including those generated by a previous multiple imputation procedure.[18]

Cox models were initially adjusted for age, sex, and race, and subsequently for additional potential confounders, including measures of body size, smoking habit, diabetes, blood pressure and antihypertensive medications, lipids and lipid-lowering treatment, serum albumin, and self-reported health status. Subsequent models examined the impact of additional factors that could serve as confounders, but might also act to mediate CML's effects. These consisted of C-reactive protein (CRP), eGFR, and the urine albumin/creatinine ratio (UACR). To test for interactions with key covariates, appropriate cross-product terms were entered using continuous levels where feasible. Sensitivity analyses examined the influence of excluding participants with diabetes, CKD (eGFR <60 mL/min/1.73 m^2), macroalbuminuria (UACR>30 mg/mmol), and hemorrhagic stroke.

All analyses were performed with STATA, version 11.0 (College Station, TX), or R version 2.13.0 (http://www.r-project.org). Two-tailed $p<0.05$ was considered statistically significant.

Results

The baseline characteristics of the study cohort are presented in Table 1, and contrasted with participants excluded for lack of CML or covariate data. Participants in the study sample were younger, more often women and reported better health status, but had similar levels of other cardiovascular risk factors, as compared with excluded participants.

Cross-sectional associations of serum CML with baseline covariates are detailed in Table 2. Serum CML showed modest positive correlations with age, systolic blood pressure, HDL-cholesterol, serum albumin and creatinine, and UACR, while exhibiting modest negative correlations with anthropometric parameters, fasting glucose and insulin, LDL-cholesterol and triglycerides, physical activity, and eGFR. Circulating CML levels were higher among participants receiving lipid-lowering medication, those with eGFR<60 mL/min/1.73 m^2 and micro- or macroalbuminuria, and those reporting fair/poor health status or previous unintentional weight loss.

During a median follow-up of 9.1 years, 625 participants experienced at least one incident cardiovascular event (CHD, n=417; stroke, n=274; both, n=66). Adjusted cubic spline analysis was consistent with a linear association between serum CML and incident CHD or stroke (Figure 1). The relative risk estimates for continuous increases in serum CML concentration are given in Table 3. After adjustment for potential confounding (model 2), every SD increase (0.99 pmol/l) in serum CML was associated with a significant 11% increase in the relative risk of the primary composite endpoint. This was not meaningfully altered after additional adjustment for CRP, eGFR and UACR, or when HOMA2-IR was entered as a covariate. As also shown in Table 3, risk estimates were consistent for the individual components, CHD and stroke, both after adjustment for potential confounders and for putative causal intermediates.

There was no evidence of effect-modification of the association of CML with cardiovascular events by age, sex, race, diabetes, BMI, or eGFR (all $p>0.65$). A significant interaction by UACR was detected (continuous CML × continuous UACR, $p<0.001$), but this did not persist after removal of extreme upper values of UACR ($p=0.57$ after exclusion of n=38 participants with macroalbuminuria). Given its tenuous nature, we did not deem this interaction sufficiently compelling to pursue further. Sensitivity analyses showed similar effect estimates after exclusion of participants with macroalbuminuria, eGFR<60 mL/min/1.73 m^2 and diabetes, as well as following exclusion or censoring of hemorrhagic stroke.

Discussion

This investigation is to our knowledge the first prospective study to examine the association of serum CML with incident CHD or stroke in a general population-based sample of older adults. It is also the largest study to date to evaluate the relationship between CML and clinical outcomes of any kind. The current analyses document that serum CML is associated

with incident cardiovascular events in older men and women independent of an array of potential confounders and/or mediators.

The significant association of serum CML with incident cardiovascular events independent of traditional atherosclerosis risk factors is compatible with the well-documented vascular effects of AGEs in experimental studies.[4] As a dominant AGE in human tissues,[10, 11] and one whose circulating levels correlate strongly with those of other AGEs,[14, 15, 27] CML is deemed an attractive measure of overall AGE burden, vascular and otherwise.

The adverse vascular consequences demonstrated experimentally for AGEs fall broadly into receptor-independent and receptor-dependent effects.[1] The first encompass the modification of extracellular matrix proteins, which results in heightened vascular stiffness.[4] Receptor-independent effects also involve modification of LDL-cholesterol, impeding its removal from the subendothelial space.[4] The second category entails receptor-dependent effects mediated by AGE binding to RAGE, which triggers intracellular pathways that promote formation of reactive oxygen species, deplete nitric oxide, and foster activation of NFkB.[1] Together, these pathways promote expression of pro-inflammatory cytokines and cell-adhesion molecules, which play critical roles in atherogenesis.[4] The aggregate impact of AGEs on the aorta, as well as the coronary and cerebral circulations, would explain the comparable associations of CML with CHD and stroke here, driven by large-artery atherothrombosis and its sequelae and, especially in the brain, by small-artery disease.[4]

The current study took care to account for the potential impact of CKD in assessing the association of CML with incident CHD and stroke. Renal disease is an important determinant of AGE formation, marked as it is by the accumulation of reactive carbonyl species and increased oxidative stress.[28] Kidney disease also reduces clearance of circulating AGEs,[28] both albumin-bound and, especially, small AGE peptides and free adducts.[29] Consistent with the relevance of chronic kidney disease to circulating AGE concentrations, CML levels were correlated with eGFR and albuminuria in the present analyses. Adjustment for the latter measures, however, did not substantially attenuate the association of CML with CHD and stroke. While there was a suggestion of interaction by albuminuria, its lack of gradation across increasing UACR levels when a small number of participants with extreme upper values were excluded, makes this finding suspect. Future studies with larger numbers of individuals with severe albuminuria are required to explore the influence, if any, of macroalbuminuria on CML's relationship with cardiovascular events.

As in previous epidemiological studies that have detected associations between AGEs/CML and mortality,[7, 8, 12, 13] the present findings show no obvious differences according to diabetes status. Moreover, consistent with previous reports,[7, 30, 31] no positive correlations were observed between serum CML and measures of glycemia. Instead, weak negative correlations with fasting glucose and insulin were apparent. Given that diabetes is a well-established risk factor for AGE formation,[1] these findings could relate to the negative correlation of CML with adiposity, whose basis remains uncertain.[30] Furthermore, the lack of positive correlations between CML and glycemia could reflect endogenous glycoxidation

or lipid peroxidation reactions,[31–33] and exogenous dietary intake,[27, 34] rather than hyperglycemia-mediated glycation, as principal sources of CML formation.

The results of this study have important clinical implications. The moderate association of CML with incident cardiovascular events indicates that this biomarker would be of limited value for risk prediction. But the independent link uncovered suggests that targeting older adults with elevated CML levels with AGE-lowering interventions could have substantial therapeutic benefits, particularly since elders' high baseline cardiovascular risk[35] yields larger absolute risk reductions. Moreover, the potential of such interventions could extend beyond that suggested by this individual biomarker, because CML is strongly correlated with other major AGEs[14, 15, 27] whose levels would be lowered concomitantly.

As relates to AGE/CML lowering strategies, the extent to which dietary AGEs lead to deleterious health consequences in humans has been a matter of controversy,[36] but dietary intervention studies have shown AGE-rich meals to be associated with higher circulating levels of AGEs, and measures of oxidative stress, inflammation, and insulin resistance.[37, 38] Likewise, pharmacological approaches with AGE cross-link breakers, AGE inhibitors, and dietary AGE binders have shown improvements in vascular function,[39, 40] renal function,[41] and metabolic or inflammatory derangements[42] in small intervention studies. Hence, our findings suggest that studies of dietary AGE restriction or pharmacological interventions targeted to older adults with elevated CML levels could identify effective strategies for CVD reduction in this vulnerable population.

Our study has several limitations. Because CML measurements were available in generally healthier CHS survivors, the results do not necessarily apply to more diseased older populations. An additional limitation is that while CML correlates strongly with other AGEs, it necessarily constitutes a partial marker of global AGE burden. The extent to which other major AGEs, or composite indices thereof, may provide better measures of CVD risk in older adults than CML alone will require further investigation. This question, however, has been previously evaluated in a moderate-sized cohort with type 1 diabetes, where plasma CML, carboxyethyl-lysine, and pentosidine exhibited comparable associations with incident CVD and mortality, and there was no apparent incremental value when the three AGEs were combined.[15] Another key limitation is the lack of data on dietary AGE/CML intake in this population, which would have allowed determination of the contribution of exogenous sources to circulating CML levels and their consequences. Unfortunately, a well-validated catalog of the AGE content of specific foods has yet to be developed, owing to the need to employ liquid chromatography/mass spectrometry for such purposes.[43] Finally, the study cohort lacked measurement of glycated hemoglobin, which would have provided a comparative measure of longer-term glycemic burden than that available from fasting glucose. Previous studies, however, have found no correlation between serum CML and glycated hemoglobin concentration in cohorts with[7, 31] or largely without[30] diabetes.

In conclusion, the current findings demonstrate for the first time that serum CML is associated with an increased risk of CHD and stroke in older adults. This prospective association proved independent of a range of traditional atherosclerosis risk factors, including measures of kidney function. The relative modest strength of the overall

association indicates that the value of CML measurement does not lie in improvement of risk prediction, but potentially in the identification of individuals who might benefit from AGE-countering therapies. Additional studies are required to define the role of dietary AGE intake to circulating CML levels and associated CVD risk, and to explore the impact of macroalbuminuria on CML's association with cardiovascular events. Nevertheless, these findings provide impetus for clinical trials to assess the efficacy of dietary or pharmacological interventions to reduce cardiovascular events in elders with elevated CML levels.

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Highlights

- Serum levels of the advanced glycoxidation endproduct CML were measured in a large community-based cohort of older adults.
- CML was positively correlated with age, blood pressure, lipid treatment, worse health, prior weight loss, and albuminuria.
- Negative correlations were seen for CML and adiposity, physical activity, LDL-C, insulin resistance, and eGFR.
- CML was associated with higher risk of incident CHD and stroke independent of multiple potential confounders and mediators.

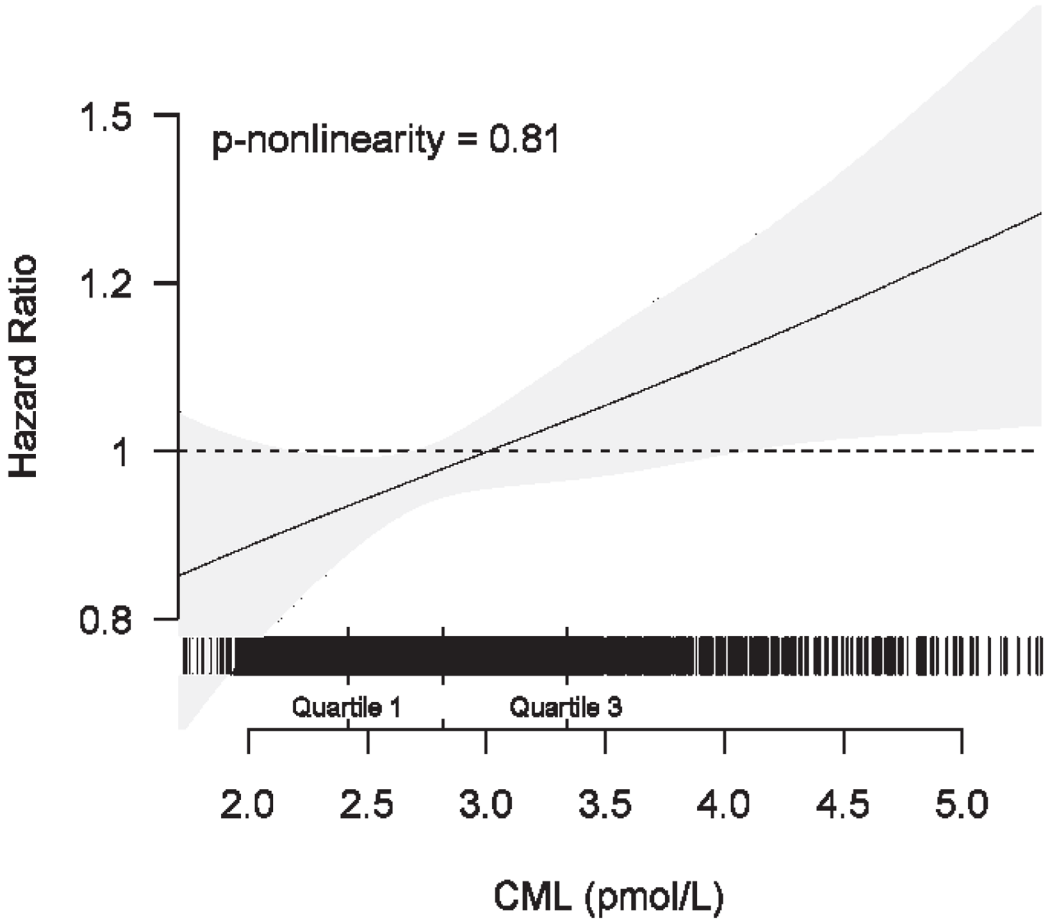

Figure 1.

Spline regression graph depicting the adjusted association of continuous CML concentration and cardiovascular events. The horizontal band above the x-axis represents individual participants (vertical lines) with corresponding values for serum CML. The model adjusts for age, sex, race, BMI, systolic blood pressure, antihypertensive medication, diabetes, smoking status, log(triglycerides), serum albumin, and self-reported health status.

Table 1

Baseline Characteristics* of Study Cohort and Excluded Participants

Covariates	Study Cohort (n=2,111)	Excluded Cohort† (n=621)
Age, years	78±5	80±6
Women	1,359(64.4)	450(72.5)
Black	344(16.3)	113(18.2)
BMI, kg/m^2	27.0±4.6	26.3±5.3
Waist-hip ratio	0.95±0.10	0.93±0.10
Systolic blood pressure, mm Hg	137±20	138±20
Diastolic blood pressure, mm Hg	71±11	70±13
Antihypertensive medication	1,004(47.6)	241(47.8)
Fasting glucose, mmol/l	5.8±1.6	5.8±1.6
Fasting insulin, mU/l	7.6(5.1–11.6)	7.1 (4.4–11.2)
HOMA2-IR	0.9(0.6–1.3)	0.8(0.5–1.3)
Diabetes	324(15.3)	92(14.8)
LDL-C, mmol/l	3.3±0.9	3.4±0.9
HDL-C, mmol/l	57±16	57±16
Triglycerides, mmol/l	1.3(1.0–1.8)	1.3(1.0–1.8)
Lipid-lowering medication	167(7.9)	28(5.6)
Albumin, μmol/l	5.5±0.4	5.5±0.4
Current smokers	161(7.6)	56(9.0)
Alcohol use ≥ 7 drinks/wk	271(12.8)	61(9.8)
Estrogen replacement (women)	238(17.5)	47(12.6)
Physical activity, kcal/wk	803(270–1755)	510(90–1350)
Fair/poor self-reported health status	384(18.2)	210(33.8)
Unintentional weight loss>4.5 kg	100 (5.0)	31(6.8)
eGFR (cystatin-based), ml/min/1.73 m^2	73±19	74±21
eGFR <60 ml/min/1.73 m^2	465(22.0)	104(16.7)
UACR, mg/mmol	1.0(0.6–2.0)	1.2(0.7–2.7)
Microalbuminuria	234(11.3)	26(17.9)
Macroalbuminuria	38(1.8)	3(2.1)
CRP, nmol/l	21.9(9.5–45.7)	23.8(10.5–44.8)

*n(%), mean±SD or median(interquartile range)

†Involves participants lacking CML (n=565) or imputable covariates (n=56).

Table 2

Relationship of Serum Carboxymethyl-Lysine with Baseline Covariates

Covariates	**Carboxymethyl-Lysine**	
	***Correlation Coefficient or Mean**[*] (95% CI)*	***P***
Age	0.11	<0.001
Sex		0.94
Women (n=1,359)	2.88(2.84–2.92)	
Men (n=752)	2.88(2.83–2.94)	
Race-ethnicity		0.066
Black (n=344)	2.95(2.87–3.04)	
Non-Black (n=1,767)	2.87(2.83–2.90)	
BMI	−0.15	<0.001
Waist-hip ratio	−0.11	<0.001
Systolic blood pressure	0.04	0.041
Diastolic blood pressure	−0.02	0.31
Antihypertensive medication		0.097
Yes (n=1,004)	2.91(2.86–2.96)	
No (n=1,107)	2.85(2.81–2.90)	
Fasting glucose	−0.06	0.012
Fasting insulin	−0.09	<0.001
HOMA2-IR[†]	−0.14	<0.001
Diabetes		0.312
Yes (n=324)	2.84(2.75–2.93)	
No (n=1,787)	2.89(2.85–2.93)	
LDL-C	−0.06	0.001
HDL-C	0.04	0.014
Triglycerides	−0.04	0.021
Lipid-lowering medication		0.003
Yes (n=167)	3.06(2.93–3.18)	
No (n=1,944)	2.87(2.83–2.90)	
Albumin	0.07	<0.001
Smoking status		0.11
Current (n=161)	2.79(2.67–2.91)	
Never/Ever (n=1,950)	2.89(2.85–2.92)	
Alcohol use		0.041
≥ 7 drinks/wk (n=271)	2.81(2.63–2.99)	
< 7 drinks/wk (n=1,840)	2.90(2.86–2.95)	
Estrogen replacement (women)		0.37
Yes (n=238)	2.84(2.73–2.94)	

Covariates	**Carboxymethyl-Lysine**	
	Correlation Coefficient or Mean* *(95% CI)*	***P***
No (n=1,121)	2.89(2.85–2.92)	
Physical activity (log-kcal/wk)	−0.06	0.004
Self-reported health status		0.002
Fair/poor (n=384)	3.00(2.92–3.09)	
Excellent/very good/good (n=1,727)	2.85(2.82–2.89)	
Unintentional weight loss>4.5 kg		0.012
Yes (n=100)	3.12(2.93–3.32)	
No (n=1,885)	2.87(2.84–2.91)	
eGFR (cystatin-based)	−0.22	<0.001
CKD		<0.001
eGFR ≥60 mL/min/1.73 m^2 (n=465)	2.82(2.79–2.85)	
eGFR <60 mL/min/1.73 m^2 (n=1,646)	3.11(3.03–3.20)	
UACR	0.08	<0.001
Albuminuria		0.008
None (n=1,796)	2.87(2.84–2.90)	
Microalbuminuria (n=234)	3.18(2.82–3.59)	
Macroalbuminuria (n=38)	3.14(2.88–3.43)	
CRP	<0.01	0.98

*Geometric mean in pmol/l

†Log-transformed

Table 3

Association of Serum Carboxymethyl-Lysine with Incident Cardiovascular Events

	Composite Cardiovascular Endpoint	
	Incident Events	***Incidence Rate* (95% CI)***
N	625	35.6(32.9–38.5)
	HR per SD† (95% CI)	***p***
Model 1	1.09(1.02–1.17)	0.014
Model 2	1.11(1.03–1.19)	0.004
Model 3	1.10(1.02–1.19)	0.009
Model 4	1.10(1.02–1.19)	0.016
	Coronary Heart Disease	
	Incident Events	***Incidence Rate* (95% CI)***
N	417	22.9(20.8–25.2)
	HR per SD† (95% CI)	***p***
Model 1	1.12(1.03–1.21)	0.009
Model 2	1.13(1.04–1.23)	0.003
Model 3	1.11(1.02–1.21)	0.019
Model 4	1.11(1.01–1.22)	0.025
	Stroke	
	Incident Events	***Incidence Rate* (95% CI)***
N	274	14.1(12.6–15.9)
	HR per SD† (95% CI)	***p***
Model 1	1.10(0.98–1.22)	0.093
Model 2	1.11(0.99–1.24)	0.063
Model 3	1.13(1.01–1.26)	0.031
Model 4	1.14(1.01–1.28)	0.027

* per 1000 person-years.

† SD = 0.99 pmol/l

Model 1. Adjusted for age, sex, and race.

Model 2. Adjusted for age, sex, race, BMI, systolic blood pressure, antihypertensive medication, diabetes, smoking status, log(triglycerides), albumin, self-reported health status. Additional adjustment for fasting glucose, other lipid fractions or unintended weight loss did not meaningfully alter the effect estimates.

Model 3. Adjusted for covariates in Model 2 and log(CRP), eGFR and UACR (in subjects with available UACR, n=2,068).

Model 4. Adjusted for covariates in Model 3 and log(HOMA2-IR) (in subjects with available UACR and not receiving diabetes medication [n=1,926]).