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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.
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 2111 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.

1. Introduction

Advanced glycation/glycoxidationendproducts (AGEs) are a diverse class of highly bioactive compounds produced when

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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 end-products (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.

2. Methods

2.1. 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 5201 subjects was recruited in 1989–1990, 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 5888 initially recruited subjects, 4412 out of 4708 surviving individuals returned for the 1996–1997 examination. Among these participants, 2732 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.

2.2. 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].

2.3. 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 1996–1997. 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%.

2.4. 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].

2.5. 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 1996–1997 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