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Relationship of an advanced glycation end product, plasma carboxymethyl-lysine, with slow walking speed in older adults: the InCHIANTI study

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Abstract

Advanced glycation end products (AGEs) are bioactive molecules found in foods and generated endogenously in the body. AGEs induce cross-linking of collagen and increase the stiffness of skeletal muscle and cartilage. We characterized the relationship between a plasma AGE, carboxymethyl-lysine (CML), and slow walking speed (lowest quintile of walking speed) in older adults. Walking speed over a 4 m course was assessed in 944 adults, aged ≥65 years, in the InCHIANTI study, a population-based study of aging and mobility disability conducted in two towns in Tuscany, Italy. Participants in the highest quartile of plasma CML were at higher risk of slow walking speed (Odds Ratio [O.R.] 1.56, 95% Confidence Interval [C.I.] 1.02-2.38, $P = 0.04$) compared to those in the lower three quartiles of plasma CML in a logistic regression models adjusting for age, education, cognitive function, smoking, and chronic diseases. After exclusion of participants with diabetes, participants in the highest quartile of plasma CML were at higher risk of slow walking speed (O.R. 1.87, 95% C.I. 1.15-3.04, $P = 0.01$) adjusting for the same covariates. In older community-dwelling adults, elevated plasma CML is independently associated with slow walking speed.

Keywords

Advanced glycation end products; aging; carboxymethyl-lysine; physical performance; walking speed

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Introduction

Sarcopenia, or loss of muscle strength and muscle mass, is an important factor underlying mobility difficulties such as slow walking speed in older adults (Lauretani et al. 2003). Advanced glycation end products have been hypothesized to play a role in the pathogenesis of sarcopenia through AGE-mediated increases in inflammation and endothelial dysfunction in the microcirculation of skeletal muscle (Payne 2006) and through cross-linking of collagen in skeletal muscle (Haus et al. 2007). The connective tissue scaffold that surrounds individual muscle fibers, muscle bundles, the whole muscle plays an important role in the transfer of force from contractile units of muscle to tendon and bone. AGE concentrations in skeletal muscle of older adults is more than two-fold higher than that found in younger adults, suggesting that glycated-related cross-linking of intramuscular connective tissue may contribute to the decline in muscle function with aging (Haus et al. 2007). In addition, AGE-induced cross-linking of collagen has been shown to increase the stiffness of human articular cartilage (Verzijl et al. 2000; Verzijl et al. 2002).

Recently, it was shown that older, disabled, community-dwelling women with elevated serum carboxymethyl-lysine (CML), a dominant AGE in serum and tissues, were at higher risk of poor grip strength (Dalal et al. in press). It is not known whether older adults with high serum AGE concentrations are at higher risk of impaired physical performance and greater disability. We hypothesized that older community-dwelling adults with elevated plasma CML were at greater risk of having a slow walking speed. To address this hypothesis, we characterized the relationship between plasma CML and walking speed in a population-based study of older adults.

Subjects and Methods

The study participants consisted of men and women, aged 65 and older, who participated in the Invecchiare in Chianti, “Aging in the Chianti Area” (InCHIANTI) study, conducted in two small towns in Tuscany, Italy. The rationale, design, and data collection have been described elsewhere, and the main outcome of this longitudinal study is mobility disability (Ferrucci et al. 1999). Briefly, in August 1998, 1270 people aged 65 years and older were randomly selected from the population registry of Greve in Chianti (population 11,709) and Bagno a Ripoli (pop. 4,704), and of 1256 eligible subjects, 1155 (90.1%) agreed to participate. Participants received an extensive description of the study and participated after written, informed consent. The study protocol complied with the Declaration of Helsinki and was approved by the Italian National Institute of Research and Care on Aging Ethical Committee. The plan for additional laboratory and data analyses were approved by the Institutional Review Board of the Johns Hopkins University School of Medicine.

Of the 1155 participants ≥65 years seen at enrollment, 1055 (91.3%) participated in the blood drawing. There were 944 (81.7%) participants at enrollment with both plasma carboxymethyl-lysine and 4 m walking speed available for this analysis. The subjects who did not participate in the blood drawing were generally older and had greater comorbidity than the subjects who participated in the blood drawing, as reported elsewhere (Schrager et al. 2007). None of the participants were taking dietary supplements.

Data collection consisted of a home interview and a medical evaluation at the study clinic, which took place within 21 days after the home interview. Demographic information was collected, including sociodemographic variables (age, sex, and years of education), smoking history, and use of medications. All participants were examined by a trained geriatrician, and diseases were ascertained according to standard, pre-established criteria and algorithms based upon those used in the Women’s Health and Aging Study for coronary heart disease, congestive

heart failure, peripheral artery diseases, diabetes mellitus, chronic obstructive pulmonary disease, and osteoarthritis (Guralnik et al. 1995). Weight was measured using a high-precision mechanical scale. Standing height was measured to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). BMI was categorized as underweight (<18.5 kg/m^2), normal range (18.5-24.9 kg/m^2), overweight (≥25-29.9 kg/m^2) and obese (≥30 kg/m^2) according to World Health Organization criteria (James et al. 2001). Mini-Mental Status Examination (MMSE) was administered at enrollment, and an MMSE score <24 was considered consistent with cognitive impairment (Folstein et al. 1975). Renal insufficiency was defined as estimated glomerular filtration rate of <60 mL/min/1.73 m^2 using the four-variable Modification of Diet in Renal Disease Study equation of Levey and colleagues (Levey et al. 1999)

In the 4-meter walking test, the participants were instructed to walk at their normal pace over a 4 meter distance, were repeated twice and the average time was used (Bandinelli et al. 2006). Participants were categorized as having slow walking speed if they were in the slowest quintile of walking speed, which in this population was <0.79 m/sec.

Blood samples were collected in the morning after a 12-h fast. Aliquots of serum and plasma were immediately obtained and stored at -80° C. The measure of plasma AGEs in this study was plasma carboxymethyl-lysine (CML). CML is a dominant circulating AGE, one of the best characterized of all the AGEs, and a dominant AGE in tissue proteins (Reddy et al. 1995). CML was measured using a competitive enzyme-linked immunosorbent assay (ELISA) (AGE-CML ELISA, Microcoat, Penzberg, Germany) (Boehm et al. 2004). This assay has been validated (Zhang et al. 2005), is specific, and shows no cross-reactivity with other compounds (Boehm et al. 2004). The minimum level of detectability of the assay is 5 ng/mL (Boehm et al. 2004), which is below the concentration that has been found in human studies. The intra-assay and inter-assay coefficients of variation were both <5%.

Variables are reported as medians (25th, 75th percentiles) or as percentages. Plasma CML was divided into quartiles, and the cut-off at the highest quartile was 424 ng/mL. Age and BMI were used as categorical variables because the relationship between age and BMI, respectively, with slow walking speed was not linear. Logistic regression models were used to examine the relationship between plasma CML and other risk factors with slow walking speed. Covariates that were significant in univariate analyses were included in the final multivariate models. All analyses were performed using SAS (v. 9.1.3, SAS Institute, Inc., Cary, NC) with a statistical significance level set at P <0.05.

Results

The demographic, anthropometric, and disease characteristics of participants with and without slow walking speed are shown in Table 1. Participants with slow walking speed were older and more likely to be female, obese, not currently smoking, with MMSE score <24, and with depression, hypertension, congestive heart failure, peripheral artery disease, stroke, diabetes, and renal insufficiency compared with participants without slow walking speed. Plasma CML concentrations were higher in participants with slow walking speed compared to those without slow walking speed. There were no significant differences in the proportion of angina or cancer among participants with and without slow walking speed.

Participants in the highest quartile of plasma CML had greater odds of slow walking speed in separate multivariate logistic regression models, adjusted for age and sex, and additionally for education, smoking, MMSE, and for chronic diseases, respectively. The association between plasma CML and slow walking speed remained significant in similar models after excluding participants with diabetes.

Discussion

The present study shows that elevated plasma CML is independently associated with slow walking speed in older community-dwelling adults. To our knowledge, this is the first study to show an association between elevated AGEs and impaired physical performance in older, community-dwelling men and women. This observation is consistent with the hypothesis that AGEs play a role in sarcopenia (Payne 2006). Increased AGEs may contribute to increased stiffness in muscle tissue and reduced viscoelastic properties of muscle and thus impair muscle function (Haus et al. 2007). In rats, AGEs accumulate in skeletal muscle with aging (Snow et al. 2008). In a rabbit model, cross-linking of collagen by nonenzymatic glycation increased the structural stiffness of Achilles tendon (Reddy 2004). Elevated AGEs are associated with increased arterial stiffness (Schram et al. 2005; Semba et al. 2008) and bone rigidity (Vashisth et al. 2001; Hernandez et al. 2005; Tang et al. 2007) which is thought to occur through cross-linking of collagen. Increased levels of CML have been described in skeletal muscle of healthy middle-aged men who reported weight increase compared to men who maintained stable weight (de la Maza et al 2008). In addition, immunostaining of skeletal muscle for CML was co-localized with immunostaining for markers of oxidative injury (8-hydroxy-deoxyguanosine and 4-hydroxy-2-nonenal) and inflammation (tumor necrosis factor-α).

AGEs are a potentially modifiable risk factor, since the diet is a major source of exogenous AGEs (Goldberg et al. 2004). The Western diet is rich in AGES, especially in foods that are processed at high temperatures. Serum CML concentrations show a moderate correlation with dietary intake of AGEs in older adults (Uribarri et al. 2007), and serum CML concentrations vary with dietary intake of AGEs (Negrean et al. 2007). Elevated serum CML concentrations have been associated with low grip strength (Dalal et al, 2009).

A limitation of this study is that causality cannot be strongly inferred from this cross-sectional study. It is possible that older adults with slow walking speed were less physically able to have access to a more healthy diet, i.e., greater intake of fruits and vegetables and lower intake of foods processed at very high temperatures. The relationship between serum AGEs and changes in walking speed needs to be examined in the future in large, prospective studies to determine whether elevated serum AGEs predict a decline in walking speed. An important consideration in longitudinal studies is the competing risk of mortality, since older adults with elevated serum AGEs are at an increased risk of all-cause and cardiovascular disease mortality (Semba et al. 2009).

In the present study, the relationship between elevated CML and slow walking speed remained strong even after exclusion of participants with diabetes. The relationship between AGEs and adverse clinical outcomes has mostly been studied in patients with diabetes and/or end-stage renal disease (Basta et al. 2004; Bohlender et al. 2005). Recent investigations show that elevated AGEs are associated with arterial stiffness (Semba et al. 2008) and cardiovascular mortality (Semba et al, 2009) in persons without diabetes. These recent findings together suggest that elevated AGEs are an important independent risk factor for adverse aging-related outcomes in older, community-dwelling adults.

In conclusion, older adults with elevated plasma CML, an advanced glycation end product, had greater risk of slow walking speed. Further studies are needed to determine whether elevated circulating AGEs are predictive of a decline in walking speed in older adults and to corroborate these findings in other cohort studies of aging.

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Table 1

Demographic and Health Characteristics of Adults, Aged ≥65 Years, in the InCHIANTI Study in Lowest versus Upper Four Quintiles of Walking Speed

Characteristic[1]		Lowest Quintile of Walking Speed (n = 188)		Upper Four Quintiles of Walking Speed (n = 756)		*P*
		N	% or Median (25th, 75th percentile)	N	% or Median (25th, 75th percentile)	
Age, years	65-69	18	6.5	259	93.5	<0.0001
	70-74	22	8.4	239	91.6	
	75-79	38	19.5	157	80.5	
	80-84	41	41.0	59	59.0	
	85-89	40	54.8	33	45.2	
	≥90	29	76.3	9	23.7	
Sex	Male	52	12.5	364	87.5	<0.0001
	Female	136	25.8	392	74.2	
Education, years		188	4.0 (3.0, 5.0)	756	5.0 (4.0, 6.0)	<0.0001
Smoking status	Never	138	24.6	423	75.4	<0.0001
	Former	32	12.5	224	87.5	
	Current	18	14.2	109	85.8	
Body mass index (kg/m^2)	<18.5	0	0	2	100	0.13
	18.5-24.9	45	17.7	210	82.3	
	25.0-29.9	72	17.0	352	83.0	
	≥30	59	23.8	189	76.2	
Plasma CML (ng/mL)		188	375 (294, 447)	756	343 (287, 413)	0.004
Plasma CML, top quartile (%)		61	32.4	168	22.2	0.003
Mini-Mental Status Exam (MMSE) score <24		101	53.7	150	19.8	<0.0001
Depression		70	41.9	120	16.0	<0.0001
Hypertension		112	59.6	343	45.4	0.0005
Angina		11	5.8	34	4.5	0.43
Congestive heart failure		26	13.8	19	2.5	<0.0001
Peripheral artery disease		19	10.1	33	4.4	0.002

Characteristic[1]	Lowest Quintile of Walking Speed (n = 188)		Upper Four Quintiles of Walking Speed (n = 756)		*P*
	N	% or Median (25th, 75th percentile)	N	% or Median (25th, 75th percentile)	
Stroke	20	10.6	23	3.0	<0.0001
Diabetes mellitus	35	18.6	81	10.7	0.003
Cancer	15	8.0	45	5.6	0.31
Renal insufficiency	45	24.2	91	12.0	<0.0001

[1]Median (25th, 75th percentile) for continuous variables. Row percentages are shown for subcategories of age, sex, smoking, and body mass index. Percentages are shown for specific conditions.

Table 2

Multivariate logistic regression models of the relation of plasma CML with slow walking speed[1] in adults, aged ≥65 years, in the InCHIANTI Study

		Model adjusted for age, sex			Model adjusted for age, sex, education, smoking, MMSE			Model adjusted for age, education, smoking, MMSE, depression, and chronic diseases[2]		
		OR	**95% CI**	***P***	**OR**	**95% CI**	***P***	**OR**	**95%CI**	***P***
Plasma CML[3]	**All participants (n = 944)**	1.51	1.01-2.25	0.04	1.52	1.01-2.28	0.04	1.60	1.02-2.52	0.04
	Participants without diabetes (n = 828)	1.76	1.13-2.73	0.01	1.75	1.12-2.74	0.01	1.87	1.15-3.04	0.01

[1] Slow walking speed defined as the lowest quintile of walking speed.

[2] Chronic diseases were hypertension, heart failure, peripheral artery disease, stroke, diabetes, and renal insufficiency.

[3] Odds ratios expressed per for highest quartile of plasma CML versus lower three quartiles in separate logistic regression models in which slow walking speed is the dependent variable.