Elevated Serum Carboxymethyl-Lysine, an Advanced Glycation End Product, Predicts Severe Walking Disability in Older Women: The Women’s Health and Aging Study I

Kai Sun, Richard D. Semba, Linda P. Fried, Debra A. Schaumberg, Luigi Ferrucci, and Ravi Varadhan

1 Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA
2 Mailman School of Public Health, Columbia University, New York, NY 10032, USA
3 Division of Preventive Medicine, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02215, USA
4 Longitudinal Studies Section, Clinical Research Branch, National Institute on Aging, Baltimore, MD 21225, USA
5 Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA

Correspondence should be addressed to Richard D. Semba, rdsemba@jhmi.edu

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Advanced glycation end products (AGEs) have been implicated in the pathogenesis of sarcopenia. Our aim was to characterize the relationship between serum carboxymethyl-lysine (CML), a major circulating AGE, and incident severe walking disability (inability to walk or walking speed \(< 0.4 \text{ m/sec}\) over 30 months of followup) in 394 moderately to severely disabled women, \(\geq 65\) years, living in the community in Baltimore, Maryland (the Women’s Health and Aging Study I). During followup, 154 (26.4%) women developed severe walking disability, and 23 women died. Women in the highest quartile of serum CML had increased risk of developing severe walking disability in a multivariate Cox proportional hazards model, adjusting for age and other potential confounders. Women with elevated serum CML are at an increased risk of developing severe walking disability. AGEs are a potentially modifiable risk factor. Further work is needed to establish a causal relationship between AGEs and walking disability.

1. Introduction

Mobility difficulties are common among older adults and are associated with poor quality of life [1], increased need for care, and are predictive of death [2–4]. Understanding the processes that lead to disability is important in order to develop strategies to prevent or delay disability in older adults. Lifestyle factors that may influence the pathway to disability include diet. Diet has been incompletely characterized in relation to the development of disability. Recent studies suggest that advanced glycation end products (AGEs), which are active biomolecules formed by the non-enzymatic covalent binding of sugars with proteins and other molecules, may be related to muscle strength and physical performance [5, 6]. The western diet is high in AGEs, which are formed in high concentrations in foods that are prepared at high temperatures. Thus, some foods are considered an important exogenous source of AGEs. AGEs are thought to be absorbed in the process of digestion, circulate in the blood, and can be deposited in different organs and tissues [7].

Sarcopenia, or loss of muscle strength and muscle mass, is an important factor underlying mobility difficulties such as slow walking speed in older adults [8]. Older adults have increased cross-linking of collagen and deposition of AGEs in skeletal muscle [9]. In aging animals, cross-linking of collagen is associated with increased muscle stiffness, reduced muscle function [10, 11], and accumulation of AGEs...
AGEs may also play a role in sarcopenia through upregulation of inflammation and endothelial dysfunction in the microcirculation of skeletal muscle through the receptor for AGES, or RAGE [13]. AGE-induced cross-linking of is collagen elevated in older adults [14] and has been shown to increase the stiffness of human articular cartilage [15].

The relationship between circulating AGES and the development of disability has not been characterized in older adults. We hypothesized that women with elevated serum carboxymethyl-lysine (CML), an advanced glycation end product, had an increased risk of developing severe walking disability. To address this hypothesis, we examined the relationship between serum CML concentrations and severe walking disability among older women living in the community.

2. Subjects and Methods

2.1. Study Population. Subjects in this study were women who participated in the Women’s Health and Aging Study I (WHAS I), a population-based study designed to evaluate the causes and course of physical disability in older disabled women living in the community. WHAS I participants were recruited from an age-stratified random sample of women aged 65 years and older selected from Medicare enrollees residing in 12 contiguous-zip-code areas in Baltimore [16]. Women were screened to identify self-reported physical disability that was categorized into four domains. The domains of disability were ascertained in a 20- to 30-minute home interview that included questions related to (1) mobility and exercise tolerance, that is, walking for a quarter of a mile, walking up 10 steps without resting, getting in and out of bed or chairs; (2) upper extremity function, that is, raising your arms up over your head, using your fingers to grasp or handle, lifting or carrying something as heavy as ten pounds; (3) higher functioning tasks (a subset of instrumental activities of daily living, not including heavy housework, that is, using the telephone, doing light housework, preparing your own meals, shopping for personal items); (4) basic self-care tasks (a subset of nonmobility dependent activities of daily living, i.e., bathing or showering, dressing, eating, using the toilet). WHAS I enrolled the one-third most disabled women ages 65 and older, those with disability in two or more domains. Of the 1409 women who met study eligibility criteria, 1002 agreed to participate in the study in 1992. There were no major differences in sociodemographic or reported health characteristics between eligible participants and those who declined to participate [16].

At the 12-month follow-up visit (as baseline for the present study), 879 women returned for follow-up, of which 580 received a blood draw. CML was measured in 531 women who had walking speed measurements available. 119 had severe walking disability, 412 did not have severe walking disability, and of whom 394 who had at least one follow-up visit thereafter were included in the present study. Laboratory measurements of serum CML were done at the 12-month follow-up visit rather than at enrollment because of a greater availability of serum aliquots from this visit.

2.2. Data Collection. Standardized questionnaires were administered in the participant’s home by trained interviewers. Race was assessed in a questionnaire as black, white, or other, current smoking as yes or no, and education as 0–8, 9–11, 12, or more than 12 years as the highest level of formal education achieved. Two weeks later, a trained registered full-time study nurse practitioner examined each study participant in her home, using a standardized evaluation of physical performance and physical exam. Approximately 75% of women also consented to phlebotomy performed during a separate visit by a trained phlebotomist who followed a standardized protocol. The definitions for the chronic diseases reported in this study were adjudicated by WHAS coinvestigators based on standardized algorithms that combined information from the questionnaire, physical examination, and physician contact [16]. The Mini-Mental State Examination (MMSE) was administered at enrollment. Further details on the methods and sampling design of the WHAS studies are published elsewhere [16].

The participant was asked to walk over a 4-meter course for each follow-up visit. Participants were instructed to stand with both feet at the starting line and to start walking after a specific verbal command. Timing began when the command was given. In this test, the subject could use a cane, a walker, or other walking aid, but not the aid of another person. The times to complete the first meter and the entire path were recorded. The test was repeated three times, twice at the woman’s usual pace, and once at her fastest possible pace. The speed of the faster of the two usual-pace walks was used in the analyses. The length of the walk expressed in meters divided by the time in seconds was used to calculate the average walking speed [16]. Women were categorized as having severe walking disability based upon being unable to walk or having a walking speed < 0.4 m/sec [17]. The 0.4 m/sec cut-off point was approximately at the top of the lowest quartile in the WHAS population at baseline [18] and has been shown to predict functional dependence [19]. Demographic characteristics, self-rated health, and information about appetite and eating were measured in the WHAS questionnaires. Chronic diseases were adjudicated by WHAS coinvestigators based on the questionnaire, physical examination, and physician contact [16]. Vital status was determined through matching with the National Death Index from the 12-month follow-up visit, 1993–96 through the end of 2000. The study protocol was adherent to the Declaration of Helsinki. The Johns Hopkins University Institutional Review Board approved the study protocol, and written informed consent was obtained from all participants.

2.3. Laboratory Studies. Nonfasting blood samples were obtained by venipuncture between 9 AM and 2 PM. Blood samples were delivered to Quest Diagnostics Laboratories (formerly Ciba-Corning Laboratories, Baltimore, MD) on the day of blood drawing for complete blood count and creatinine measurements. Serum creatinine was measured using the Jaffe method. Processing, aliquoting, and freezing were carried out at the Core Genetics Laboratory of the Johns
was defined as estimated glomerular filtration rate of 
and one of the dominant AGES in tissue proteins [20]. CML 
was measured using a competitive ELISA (AGE-CML ELISA, 
Microcoat, Penzberg, Germany) [21]. This assay has been 
validated [22], is specific, and shows no cross-reactivity with 
other compounds [21]. Measurements were performed in 
duplicate according to the protocol of the manufacturers, 
and the results were averaged. The within-assay and between-
assay coefficients of variation for serum CML were 3% and 
4%, respectively.

2.4. Statistical Analysis. Continuous variables were com-
pared using Wilcoxon rank-sum test. Categorical variables 
were compared using chi-square tests. Body mass index 
(BMI) was categorized as underweight (<18.5 kg/m²), nor-
mal range (18.5–24.9 kg/m²), overweight (≥25–29.9 kg/m²) 
and obese (≥30 kg/m²) [23]. An MMSE score of <24 was 
defined as cognitive impairment [24]. Renal insufficiency 
was defined as estimated glomerular filtration rate of 
<60 mL/min/1.73 m² using the Modification of Diet in Renal 
Disease equation of Levey and colleagues [25]. Plasma 
CML was divided into quartiles, and the cut-off between 
quartiles was 452.6, 558.8, and 689.1 ng/mL. Grouped-
time Cox proportional hazards models [26] were used to 
examine the associations between serum AGES and the risk of 
developing severe walking disability because severe walking 
disability was determined at six-month intervals. The length 
of follow-up in longitudinal analyses was 30 months. Women 
who died, or refused further participation, or were lost to 
followup after a follow-up visit were censored according to 
their severe walking disability status at their last visit in the 
study.

During the 30 months following the original 12-month 
follow-up visit (as the baseline for the present study), there 
were 23 patients who died, 9 of them died after developing 
severe walking disability whereas 14 of them died with 
no severe walking disability until the last follow-up visit. 
Cox proportional hazards models were used to examine the 
associations between CML and risk of developing severe 
walking disability. As a highly significant association between 
slow walking speed and death has been previously described 
[27], it is a reasonable assumption that some women 
developed severe walking disability prior to death. For those 
who died without developing severe walking disability, first, 
the multiple imputation technique was used to impute the 
data and then the complete data was analyzed; second, the 
severe walking disability status known data only was analyzed 
with weighting by inverse probability weighting (IPW) on 
the data [28]; finally, two sensitivity analyses were applied 
to validate those two methods dealing with the incomplete 
data: (1) treating all missing data because of death as severe 
walking disability-free; (2) treating the missing data as severe 
walking disability developed [29]. The statistical program 
used was SAS 9.1 (SAS Institute, Cary, NC). The level of 
significance used in this study was P < 0.05.

3. Results

There were 394 women who did not have severe walking 
disability at baseline had serum carboxymethyl-lysine (CML) 
measurements available, and had at least one followup 
involved in the present study. One hundred fifty-four 
(26.4%) of the women developed severe walking disability 
during followup with an overall rate of severe walking 
disability of 14.8 per 100 person-years. The characteristics 
of women who did and did not develop severe walking 
disability during followup are shown in Table 1. Women 
who developed severe walking disability were older and more 
likely to be affected by congestive heart failure, peripheral 
artery disease, and diabetes. There were no significant 
differences between women who developed and those who 
did not develop walking disability by race, education, current 
smoking, MMSE score, body mass index, hypertension, 
coronary artery disease, stroke, depression, osteoarthritis, 
chronic obstructive pulmonary disease, and renal insuffi-
ciency. Compared with the group of lower three quartiles of 
CML, the patients in the group of highest quartile of CML 
were more likely to develop severe walking disability.

Univariate Cox proportional hazards models were used to 
examine the relationship between demographic and health characteristics and the development of severe walk-
ing disability (Table 2). Highest quartile of CML, older age, 
congestive heart failure, peripheral artery disease, and diabetes 
mellitus were associated with an increased risk of developing severe walking disability. Multivariable 
Cox proportional hazards model that adjusted for age, 
congestive heart failure, peripheral artery disease, diabetes 
mellitus, and renal insufficiency are shown in four scenarios 
(Table 3).

4. Discussion

The present study shows that older community-dwelling 
women with elevated serum CML are at increased risk 
of developing severe walking disability. These findings are 
consistent with a previous, cross-sectional study which 
showed that elevated plasma CML was associated with 
slow walking speed in older community-dwelling men and 
women [6]. The present study extends these findings and 
shows that elevated CML is an independent predictor of the 
development of severe walking disability.

These observations are consistent with the hypothesis 
that AGEs play a role in sarcopenia [13]. Increased AGEs may 
contribute to increased stiffness in muscle tissue and reduced 
viscoelastic properties of muscle and thus impair muscle 
function [9]. Collagen is the main structural component 
of the interstitial space of skeletal muscle and provides 
elasticity when muscles contract [30]. Aging muscle is 
characterized by an increase in pyridinium cross-linking 
of collagen, which is enzymatically derived, and the non-
enzymatic accumulation of cross-linking AGEs. In adults 
without diabetes, the concentration of pentosidine, a major 
AGE in tissues, was found in skeletal muscle at greater than 
two times concentration in older compared with younger 
adults [9].
Table 1: Characteristics of women who developed and did not develop severe walking disability in the women's health and aging study I ($N = 394$).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Incident severe walking disability ($n = 104$)</th>
<th>No incident severe walking disability ($n = 290$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age category (years) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤69</td>
<td>17.3</td>
<td>26.2</td>
<td></td>
</tr>
<tr>
<td>70–74.9</td>
<td>22.1</td>
<td>27.6</td>
<td></td>
</tr>
<tr>
<td>75–79.9</td>
<td>14.4</td>
<td>22.1</td>
<td>0.0005</td>
</tr>
<tr>
<td>80–84.9</td>
<td>14.4</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>85–89.9</td>
<td>23.1</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>≥90</td>
<td>8.7</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Race (white) (%)</td>
<td>69.2</td>
<td>75.5</td>
<td>0.21</td>
</tr>
<tr>
<td>Education &lt; 12 years (%)</td>
<td>62.1</td>
<td>60.3</td>
<td>0.75</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>15.4</td>
<td>10.0</td>
<td>0.14</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>3.2</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>27.7</td>
<td>23.2</td>
<td>0.83</td>
</tr>
<tr>
<td>25.0–29.9</td>
<td>35.1</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td>≥30</td>
<td>34.0</td>
<td>37.2</td>
<td></td>
</tr>
<tr>
<td>MMSE score &lt; 24 (%)</td>
<td>15.4</td>
<td>9.7</td>
<td>0.11</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>57.7</td>
<td>56.2</td>
<td>0.79</td>
</tr>
<tr>
<td>Coronary heart disease (%)</td>
<td>24.0</td>
<td>22.1</td>
<td>0.68</td>
</tr>
<tr>
<td>Congestive heart failure (%)</td>
<td>14.4</td>
<td>7.2</td>
<td>0.029</td>
</tr>
<tr>
<td>Peripheral artery disease (%)</td>
<td>26.0</td>
<td>15.5</td>
<td>0.018</td>
</tr>
<tr>
<td>Stroke (%)</td>
<td>1.0</td>
<td>2.8</td>
<td>0.29</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>23.1</td>
<td>11.7</td>
<td>0.005</td>
</tr>
<tr>
<td>Depression (%)</td>
<td>15.4</td>
<td>11.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease (%)</td>
<td>29.8</td>
<td>25.9</td>
<td>0.44</td>
</tr>
<tr>
<td>Renal insufficiency (%)</td>
<td>58.4</td>
<td>52.5</td>
<td>0.30</td>
</tr>
<tr>
<td>Osteoarthritis (%)</td>
<td>57.7</td>
<td>53.5</td>
<td>0.46</td>
</tr>
<tr>
<td>CML highest quartile (%)</td>
<td>33.7</td>
<td>22.4</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Tendons transfer the forces generated by muscles to bone and have elastic properties that affect the muscle tendon complex. AGEs are deposited in tendons and can affect their structural properties. In a rabbit model, cross-linking of collagen by nonenzymatic glycation increased the structural stiffness of Achilles tendon [31]. In humans, there was a sevenfold difference in concentrations of pentosidine in the patellar tendon in older compared with younger men [14]. Elevated AGEs are also associated with increased bone rigidity [32] which is thought to occur through cross-linking of collagen [33].

AGEs may also affect skeletal muscle and tendons through upregulation of inflammation via binding with RAGE [34]. The carboxymethyl-lysine (CML) adduct of AGEs has been identified as a signal-transducing ligand for RAGE, both in vitro and in vivo [35]. Ligand binding with RAGE triggers the induction of increased reactive oxygen species, activation of NADPH oxidase, and upregulation of inflammation through NF-κB and other signaling pathways [36, 37]. Inflammatory mediators that are upregulated through AGE and the NF-κB pathway include TNF-α, IL-6, and C-reactive protein [38].

A limitation of this study is that the results cannot necessarily be extrapolated to men or to less disabled women in the community. There are many different AGEs, and in the present study, CML was the only AGE that was measured in serum. However, other studies have shown that circulating CML has a moderate correlation with other AGEs in the blood such as pentosidine and methylglyoxal derivatives [39]. Serum CML concentrations show a moderate correlation with dietary intake of AGEs in older adults [39]. Serum CML concentrations have been reported to vary with dietary intake of AGES [40], but these findings have not been corroborated by others [41, 42].

5. Conclusions

Older women with elevated plasma CML, an advanced glycation end product, had greater risk of developing severe walking disability. Whether modification of dietary intake of
Table 2: Univariate Cox proportional hazard models for demographic and health characteristics and incidence of severe walking disability in the women's health and aging study I (N = 394).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariate H.R.</th>
<th>95% C.I.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤69</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>70–74.9</td>
<td>1.18</td>
<td>0.64, 2.18</td>
<td>0.60</td>
</tr>
<tr>
<td>75–79.9</td>
<td>0.93</td>
<td>0.46, 1.86</td>
<td>0.83</td>
</tr>
<tr>
<td>80–84.9</td>
<td>1.96</td>
<td>0.99, 3.89</td>
<td>0.05</td>
</tr>
<tr>
<td>85–89.9</td>
<td>2.35</td>
<td>1.28, 4.34</td>
<td>0.006</td>
</tr>
<tr>
<td>≥90</td>
<td>5.04</td>
<td>2.25, 11.26</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Race (white)</td>
<td>0.76</td>
<td>0.50, 1.16</td>
<td>0.19</td>
</tr>
<tr>
<td>Education &lt; 12 years</td>
<td>1.12</td>
<td>0.75, 1.67</td>
<td>0.58</td>
</tr>
<tr>
<td>Current smoking</td>
<td>1.59</td>
<td>0.93, 2.70</td>
<td>0.09</td>
</tr>
<tr>
<td>Body mass index (kg/m²)¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>0.98</td>
<td>0.30, 3.25</td>
<td>0.98</td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>25.0–29.9</td>
<td>0.75</td>
<td>0.45, 1.26</td>
<td>0.28</td>
</tr>
<tr>
<td>≥30</td>
<td>0.75</td>
<td>0.45, 1.27</td>
<td>0.28</td>
</tr>
<tr>
<td>MMSE &lt; 24</td>
<td>1.59</td>
<td>0.93, 2.70</td>
<td>0.09</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.04</td>
<td>0.71, 1.54</td>
<td>0.84</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>1.09</td>
<td>0.70, 1.71</td>
<td>0.70</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>1.87</td>
<td>1.08, 3.24</td>
<td>0.03</td>
</tr>
<tr>
<td>Peripheral artery disease</td>
<td>1.74</td>
<td>1.12, 2.72</td>
<td>0.01</td>
</tr>
<tr>
<td>Stroke</td>
<td>0.46</td>
<td>0.06, 3.31</td>
<td>0.44</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>1.21</td>
<td>0.80, 1.85</td>
<td>0.37</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.79</td>
<td>1.13, 2.85</td>
<td>0.01</td>
</tr>
<tr>
<td>Depression</td>
<td>1.21</td>
<td>0.70, 2.09</td>
<td>0.49</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>1.18</td>
<td>0.79, 1.75</td>
<td>0.42</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>1.22</td>
<td>0.83, 1.81</td>
<td>0.32</td>
</tr>
<tr>
<td>CML highest quartile</td>
<td>1.68</td>
<td>1.11, 2.52</td>
<td>0.01</td>
</tr>
</tbody>
</table>

¹For body mass index, 18.5–24.9 kg/m² was used as the reference category.

Table 3: Multivariate cox proportional hazard models for serum CML and development of severe walking disability, (N = 394)¹.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>H.R. ²</th>
<th>95% C.I.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) All women with missing data had multiple simulations to impute severe walking disability status prior to death.</td>
<td>1.63</td>
<td>1.06, 2.49</td>
<td>0.03</td>
</tr>
<tr>
<td>(2) Inverse probability weighting method (IPW)</td>
<td>1.56</td>
<td>1.04, 2.36</td>
<td>0.03</td>
</tr>
<tr>
<td>(3) All women with missing data treated as censored, that is, no severe walking disability prior to death.</td>
<td>1.46</td>
<td>0.95, 2.23</td>
<td>0.08</td>
</tr>
<tr>
<td>(4) All women with missing data treated as developing severe walking disability prior to death.</td>
<td>1.54</td>
<td>1.04, 2.29</td>
<td>0.03</td>
</tr>
</tbody>
</table>

¹All models adjusted for age, congestive heart failure, peripheral artery disease, diabetes mellitus, and renal insufficiency.

²Highest quartile of serum CML versus lower three quartiles.

AGEs can influence the pathway to disability in older adults is not known.

References


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