

Fat Mass Is Inversely Associated with Serum Carboxymethyl-Lysine, An Advanced Glycation End Product, in Adults^{1,2}

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Abstract

High levels of circulating advanced glycation end products (AGE) are associated with cardiovascular disease, diabetes, chronic kidney disease, and increased mortality, but factors that influence levels of circulating AGE are not well known. Our objective was to characterize the relationship between serum carboxymethyl-lysine (CML), a major circulating AGE, and body composition in adults. In a cross-sectional study, total body DXA was performed and serum CML was measured in 592 adults, aged 26–93 y, from the Baltimore Longitudinal Study of Aging. Median (25th, 75th percentile) CML concentrations were 2.26 (1.86, 2.67) $\mu\text{mol/L}$. Total fat mass [$\beta = -0.17$ (95% CI $-0.10, -0.24$); $P < 0.0001$], truncal fat mass [$\beta = -0.17$ (95% CI $-0.10, -0.25$); $P < 0.0001$], and appendicular fat mass [$\beta = -0.13$ (95% CI $-0.05, -0.20$); $P = 0.001$] per 1 SD increase were inversely associated with serum CML in separate multivariate linear regression models, adjusting for age, sex, BMI, systolic blood pressure, TG, HDL cholesterol, and renal function. Lean body mass was not independently associated with serum CML. These findings suggest that serum CML concentration is strongly affected by body fat, possibly because CML is preferentially deposited in fat tissue or because adipocytes affect the metabolism of AGE. *J. Nutr.* 141: 1726–1730, 2011.

Introduction

AGE⁶ are a heterogeneous group of bioactive molecules that are formed from the nonenzymatic glycation of proteins, lipids, and nucleic acids. AGE have been implicated in the pathogenesis of atherosclerosis, diabetes, cardiovascular disease, and chronic kidney disease (1). There are 2 major sources of exposure to AGE in humans: exogenous AGE that are found in foods, especially foods that have been processed at high temperatures, and endogenous AGE that are generated by abnormal glucose metabolism or lipid oxidation. The contribution of dietary AGE to the total pool of AGE in the body is much greater than the contribution from AGE that are endogenously generated by abnormal glucose metabolism or lipid oxidation (2).

CML, hydroimidazolone, pentosidine, and glucosepane are among the major AGE that have been studied in humans and animal models. AGE may adversely affect human health by forming covalent cross links with proteins. The resulting cross

links increase the stiffness of tissues such as the vasculature and contribute to hypertension and heart failure (3–5). AGE increase oxidative stress and inflammation by binding with the receptor for AGE (6). Ligand binding leads to generation of reactive oxygen species, increased expression of adhesion molecules, and upregulation of inflammation through the NF- κ B pathway and other signaling pathways (6).

Epidemiological studies show that older adults with elevated plasma or serum CML are at an increased risk of all-cause and cardiovascular mortality (7–9). Two studies have reported a direct correlation between dietary intake of AGE and CML concentrations in the blood (10,11). It is not known whether circulating CML concentrations could be used as a concentration biomarker for dietary intake of AGE. The levels of circulating AGE are affected by renal function, with higher serum or plasma AGE concentrations found in people with chronic kidney disease (1).

The amount of fat mass is known to influence some dietary biomarkers. For example, serum carotenoid concentrations are modified by fat mass, because carotenoids are stored preferentially in adipose tissue (12). Whether fat mass influences serum CML concentrations is not known. Adipocytes express CD36, a scavenger receptor that binds with AGE and facilitates the endocytosis and degradation of AGE (13,14). Thus, fat mass could potentially influence circulating AGE concentrations through a role in the catabolism and breakdown of AGE. The aim of our study was to determine whether differences in body

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⁶ Abbreviations used: AGE, advanced glycation end product; BLSA, Baltimore Longitudinal Study of Aging; CML, carboxymethyl-lysine; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostasis model assessment insulin resistance.

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composition were independently associated with serum CML concentrations. To address this aim, we examined the relationship between serum CML and body composition in a cross-sectional study of older adults.

Participants and Methods

Study participants. The study participants consisted of 592 men and women in the BLSA who were seen between April 2002 and August 2007. The BLSA is a prospective open cohort study of community-dwelling volunteers, largely from the Baltimore/Washington area. The study was established in 1958 to study normative aging in a volunteer cohort of healthy persons ≥ 17 y of age at study entry (15). Participants were enrolled if they were healthy at baseline (e.g. no evidence of diabetes, stroke, heart disease, or heart failure) but remain in the study if disease develops. Currently ~ 1100 men and women actively participate in the study. BLSA participants return every 2 y to the Gerontology Research Center in Baltimore, MD, for 2.5 d of medical, physiological, and psychological examinations (15). Blood pressure was measured with a mercury sphygmomanometer and values used in this study were the mean of the second and third measurements on both the right and left arms. Height, weight, and waist circumference were measured in all participants. BMI was determined as kg/m^2 .

Normal, impaired, and diabetic fasting glucose were defined as fasting plasma glucose ≤ 99 mg/dL (≤ 5.54 mmol/L), 100–125 mg/dL (5.55–9.94 mmol/L), and >125 mg/dL (>9.94 mmol/L), respectively (20). Normal, impaired, and diabetic glucose tolerance were defined as 2-h plasma glucose ≤ 139 mg/dL (≤ 7.72 mmol/L), 140–199 mg/dL (7.73–1.10 mmol/L), and ≥ 200 mg/dL (≥ 11.11 mmol/L), respectively (20). The HOMA-IR index was calculated as fasting insulin concentration \times fasting glucose concentration/22.5 (21). eGFR in $\text{mL}/(\text{min}\cdot 1.73\text{m}^2)$ was calculated using the Modification of Diet in Renal Disease equation (22).

Total body DXA was performed using the Prodigy Scanner (General Electric) and analyzed with version 10.51.006 software (General Electric). All participants provided written, informed consent. The BLSA has continuing approval from the Institutional Review Board of the MedStar Research Institute, and the protocol for the present study was also approved by the Institutional Review Board of the Johns Hopkins University School of Medicine.

Laboratory methods. Blood samples were drawn from the antecubital vein between 0700 and 0800 h after an overnight fast. Participants were sitting in a semireclining chair during oral glucose tolerance testing. Fasting blood samples were collected at baseline, after which participants drank 75 g glucose in 300 mL solution (SunDex; Fisherbrand) and blood samples were drawn 2 h after oral administration. The plasma glucose concentration was measured by the glucose oxidase method (Beckman Instruments). Plasma insulin was measured using ELISA (Alpco Diagnostics). Hemoglobin A_{1c} was measured on a BioRad Instrument. Concentrations of plasma TG and total cholesterol were determined by an enzymatic method (Abbott Laboratories ABA-200 ATC Biochromatic Analyzer). The concentration of HDL cholesterol was determined by a dextran sulfate-magnesium precipitation procedure (16). LDL cholesterol concentrations were estimated by using the Friedewald formula (17). Samples were stored continuously at -80°C until the time of analysis of serum CML. CML was measured in duplicate at the Johns Hopkins School of Medicine (R.D.S.) using a competitive ELISA (AGE-CML ELISA, Microcoat) (18). This assay has been validated (19), is specific, and shows no cross-reactivity with other compounds (18). The within assay and between-assay CV in this study were both $<5\%$, respectively.

Statistical analysis. Continuous variables were reported as mean \pm SD or median (25th, 75th percentile). Spearman correlations were used to examine correlations between selected variables. Multivariate linear regression models were used to examine the relationship between total body fat, truncal fat, and appendicular fat (per 1 SD), demographic, anthropometric, laboratory, and clinical characteristics, with serum CML, where serum CML was the dependent variable. All multivariate linear regression models were adjusted for age as a continuous variable and sex with addition of covariates that were significantly related to

tertiles of serum CML. These covariates included BMI, systolic blood pressure, TG, HDL cholesterol, and eGFR. Additional multivariate linear regression models were examined in which either fasting plasma glucose, glucose tolerance, or HOMA-IR were added to all the covariates in the final models (model 3 for each outcome). All analyses were performed using SAS (v. 9.1.3, SAS Institute) with a type I error of 0.05.

Results

Median (25th, 75th percentile) CML concentrations in the study participants were 2.26 (1.86, 2.67) $\mu\text{mol}/\text{L}$. Basic demographic, anthropometric, and other characteristics of the study participants by tertile of CML are shown in Table 1. BMI, total fat mass, truncal fat mass, lean body mass, TG, and eGFR decreased significantly across increasing tertiles of serum CML. Systolic blood pressure was significantly different across tertiles of serum CML. HDL cholesterol increased significantly across increasing tertiles of serum CML. Age, sex, race, smoking, diastolic blood pressure, LDL cholesterol, hemoglobin A_{1c} , fasting plasma glucose, glucose tolerance, fasting plasma insulin, HOMA-IR, and chronic diseases did not significantly differ by tertiles of serum CML.

Spearman correlations between serum CML and fat measures were as follows: total fat mass ($r_s = -0.29$; $P < 0.0001$), truncal fat mass ($r_s = -0.32$; $P < 0.0001$), and appendicular fat mass ($r_s = -0.22$; $P < 0.0001$), respectively. The Spearman correlation between serum CML and lean body mass was $r_s = -0.09$ ($P = 0.02$).

Separate multivariate linear regression models were used to examine the relationships between total body fat, truncal fat mass, appendicular fat mass, lean body mass, and serum CML (Table 2). After adjusting for age and sex (model 1), additionally for BMI, systolic blood pressure, TG, and HDL cholesterol, (model 2), and finally with eGFR added to the model (model 3), in respective models, total fat mass, truncal fat mass, and appendicular fat mass were significantly associated with serum CML. Lean body mass was not significantly associated with serum CML in any of the 3 models.

To gain insight into the potential effect of abnormal glucose metabolism on the relationship between total fat mass and serum CML when fasting plasma glucose, glucose tolerance, or insulin resistance was added to total fat mass and all the covariates in model 3, the results were virtually unchanged (per 1 SD increase in total fat mass): $[\beta = -0.17$ (95% CI $-0.96, -0.24$); $P < 0.0001$], $[\beta = -0.17$ (95% CI $-0.09, -0.24$); $P < 0.0001$], and $[\beta = -0.17$ (95% CI $-0.10, -0.24$); $P < 0.0001$], respectively. When fasting plasma glucose, glucose tolerance, or insulin resistance were added to truncal fat mass and all the covariates in model 3, the results were virtually unchanged (per 1 SD increase in total fat mass): $[\beta = -0.17$ (95% CI $-0.03, -0.25$); $P < 0.0001$], $[\beta = -0.17$ (95% CI $-0.10, -0.24$); $P < 0.0001$], and $[\beta = -0.18$ (95% CI $-0.10, -0.25$); $P < 0.0001$], respectively. When fasting plasma glucose, glucose tolerance, or insulin resistance were added to appendicular fat mass and all the covariates in model 3, the results were virtually unchanged (per 1 SD increase in total fat mass): $[\beta = -0.13$ (95% CI $-0.052, -0.21$); $P = 0.001$], $[\beta = -0.12$ (95% CI $-0.05, -0.20$); $P = 0.001$], and $[\beta = -0.13$ (95% CI $-0.05, -0.20$); $P = 0.001$], respectively.

Discussion

The present study shows that fat mass, as measured by DXA, is inversely associated with serum CML concentrations in older adults. To our knowledge, this is the first study to show that total

TABLE 1 Characteristics of 592 adults by tertiles of serum carboxymethyl-lysine (CML) in the Baltimore Longitudinal Study of Aging (BLSA)^{1,2}

Characteristic	Tertile of serum CML			P ³
	≤2.02 μmol/L (n = 197)	2.03–2.54 μmol/L (n = 198)	>2.54 μmol/L (n = 197)	
Age, y	62.8 (55.0, 70.8)	61.8 (54.4, 74.6)	64.7 (56.1, 74.7)	0.10
Male gender, %	55.3	45.6	50.2	0.16
Race, white, %	62.4	62.4	64.5	0.88
Current smoking, %	6.6	4.6	5.1	0.65
BMI, kg/m ²	27.7 (25.0, 30.9)	26.2 (23.5, 30.4)	25.7 (23.2, 28.5)	0.0002
Total fat mass, kg	28.9 (22.9, 37.0)	25.6 (19.3, 31.8)	22.6 (17.7, 28.4)	<0.0001
Truncal fat mass, kg	16.3 (12.0, 20.2)	13.5 (10.1, 17.5)	11.9 (8.9, 15.1)	<0.0001
Appendicular fat mass, kg	12.0 (8.8, 15.5)	10.6 (8.0, 14.2)	9.7 (7.4, 12.5)	<0.0001
Lean body mass, kg	51.1 (41.4, 59.1)	47.3 (39.2, 56.6)	48.2 (38.9, 56.1)	0.02
Systolic blood pressure, mm Hg	125 (118, 138)	122 (113, 132)	125 (116, 137)	0.04
Diastolic blood pressure, mm Hg	71 (64, 77)	69 (62, 75)	69 (64, 76)	0.22
TG, mmol/L	1.03 (0.78, 1.53)	0.93 (0.73, 1.25)	0.94 (0.72, 1.31)	0.04
Total cholesterol, mmol/L	4.92 (4.32, 5.59)	4.95 (4.27, 5.59)	3.08 (4.48, 5.72)	0.10
LDL cholesterol, mmol/L	2.95 (2.38, 3.62)	3.00 (2.41, 3.54)	3.03 (2.51, 3.67)	0.45
Hemoglobin A _{1c} , %	5.5 (5.1, 5.8)	5.4 (5.1, 5.8)	5.4 (5.1, 5.7)	0.52
Fasting plasma glucose, %				
≤5.54 mmol/L	73.6	77.7	76.7	0.51
5.55–9.94 mmol/L	21.8	20.3	21.3	
>9.94 mmol/L	4.6	2.0	2.0	
Oral glucose tolerance test, %				
≤7.72 mmol/L	71.1	76.7	76.0	0.57
7.73–11.10 mmol/L	22.3	16.2	16.8	
≥11.11 mmol/L	6.6	7.1	7.2	
Fasting insulin, pmol/L	47.9 (31.9, 70.8)	45.8 (31.2, 70.8)	42.3 (28.4, 63.2)	0.14
HOMA-IR, mU/L·mmol/L	1.59 (0.97, 2.48)	1.52 (1.00, 2.34)	1.42 (0.89, 2.19)	0.13
HDL cholesterol, mmol/L	1.37 (1.11, 1.63)	1.45 (1.19, 1.76)	1.50 (1.24, 1.86)	0.001
eGFR, mL/(min · 1.73 m ²)	79.2 (69.3, 90.7)	77.3 (65.1, 89.3)	75.6 (65.4, 88.5)	0.03
Hypertension, %	34.5	33.5	28.4	0.38
Diabetes, %	6.6	4.6	4.6	0.58
Angina, %	12.7	9.6	9.6	0.52
Myocardial infarction, %	5.0	2.0	2.5	0.18
Stroke, %	1.0	0.5	0	0.36
Heart failure, %	0	0.5	1.0	0.36
Cancer, %	8.1	9.6	12.2	0.39
Osteoarthritis, %	24.8	22.3	24.4	0.82

¹ Values are median (25th, 75th percentile) for continuous variables and percent for categorical variables.

² eGFR, estimated glomerular filtration rate; HOMA-IR, homeostasis model assessment insulin resistance.

³ Wilcoxon rank sum test used for continuous variables and chi-square test used for categorical variables.

body fat, truncal body fat, and appendicular body fat are all negatively associated with circulating CML. The strengths of this study are the large sample size of community-dwelling men and women, a database that allowed adjustment for renal function, and the standardized assessment of body composition in all participants. In addition, the database allowed for adjustment for effects of abnormal glucose metabolism by inclusion of either fasting plasma glucose or glucose tolerance in the multivariate analyses.

To put the findings of the present study into a clinical context, it is notable that the total fat mass of the participants in the BLSA ranged widely from <10 to >60 kg. Given 2 individuals of the same age and sex and with all other covariates in the multivariate analyses held equal, if the individuals differed by 3 SD in total fat mass (~30 kg), the difference in serum CML concentrations would be >0.490 mmol/L, which is a large difference when considered in the context of risk of death described in adults with elevated serum CML (7–9).

The consumption of foods that are high in AGE, such as fast foods, French fries, potato chips, and cola drinks is higher among those who are obese (23,24). The findings from the present study suggest that, because of the negative relationship of fat mass with serum CML concentrations, serum CML levels may actually be lower among the obese compared with those who are lean in spite of a higher intake of AGE-rich foods. In a study of 18 obese children and 18 lean control children, plasma CML, fructoslysine, and AGE-associated fluorescence were all significantly lower in obese children compared with lean children (25). These findings suggest that until the compartmentalization of CML in the various organ districts is fully understood, CML, and probably other serum AGE, cannot be used as a direct biomarker for dietary intake of AGE.

Whether dietary AGE have an adverse impact on human health remains highly controversial (26–28). Current major barriers to progress in the understanding of dietary AGE in human health include the lack of a comprehensive reference

TABLE 2 Separate multivariate linear regression models of relationship total fat mass, truncal fat mass, and appendicular fat mass, respectively, and serum carboxymethyl-lysine (CML) in 592 adults in the Baltimore Longitudinal Study of Aging (BLSA)¹

	Model 1, adjusted for age and sex			Model 2, adjusted for age, sex, BMI, systolic BP, TG, and HDL cholesterol			Model 3, adjusted for age, sex, BMI, systolic BP, TG, HDL cholesterol, and eGFR		
	β	95% CI	<i>P</i>	β	95% CI	<i>P</i>	β	95% CI	<i>P</i>
Total fat mass (kg) (per 1 SD increase) ²	-0.21	-0.16, -0.27	<0.0001	-0.18	-0.11, -0.25	<0.0001	-0.17	-0.10, -0.24	<0.0001
Truncal fat mass (kg) (per 1 SD increase) ³	-0.22	-0.16, -0.28	<0.0001	-0.19	-0.12, -0.26	<0.0001	-0.17	-0.10, -0.25	<0.0001
Appendicular fat mass (kg) (per 1 SD increase) ⁴	-0.16	-0.10, -0.22	<0.0001	-0.14	-0.06, -0.22	0.0005	-0.13	-0.05, -0.20	0.001
Lean body mass (kg) (per 1 SD increase) ⁵	-0.07	-0.04, 0.17	0.19	0.04	-0.09, 0.16	0.55	0.03	-0.09, 0.16	0.61

¹ BP, blood pressure; eGFR, estimated glomerular filtration rate.

² One SD of total fat mass = 10.1 kg.

³ One SD of truncal fat mass = 5.8 kg.

⁴ One SD of appendicular fat mass = 5.5 kg.

⁵ One SD of lean body mass = 10.5 kg.

database of CML in different foods, where CML has been measured using sensitive and accurate gold standard measurement techniques such as LC-MS and where careful preparation of food samples has been conducted to minimize matrix effects (29,30). ELISA is not considered appropriate for quantitative food analyses and has led to erroneous reporting of CML values in foods (30,31). There is no assessment method for dietary AGE that has been published and rigorously validated by independent groups working with different populations. The lack of a validated concentration biomarker for intake of dietary AGE remains a problem. For these reasons, it was not possible to measure dietary consumption of AGE in the present study.

The present study shows that fasting plasma glucose, oral glucose tolerance, and insulin resistance were not significant determinants of serum CML. These findings are consistent with a previous study that found no relationship between serum CML and fasting plasma glucose (32). A recent longitudinal study showed that normalization of plasma glucose levels in patients with type 2 diabetes with insulin treatment was not accompanied by any significant change in serum CML concentrations (33). In addition, there was no significant association between glycated hemoglobin and CML in the present study, which is consistent with previous reports (34,35). A limitation of the present study is that markers of inflammation were not available. No significant relationship was found between plasma CML and IL-6 or C-reactive protein in nearly 1000 adults ≥ 65 y in the InCHIANTI Study (R. D. Semba, unpublished data).

Most clinical studies of CML have been conducted in highly selected groups from clinic-based or hospital settings, i.e. patients with diabetic nephropathy, heart failure, or end-stage renal disease (1). These studies generally show an association between high levels of CML and more severe disease. However, the present population-based study did not show an association of elevated CML with either diabetes or cardiovascular disease, which suggests that the association between elevated circulating AGE is stronger in highly selected groups of patients than in a representative population of community-dwelling adults.

Because CML concentrations could potentially be influenced by the rate of protein turnover, a limitation of the study is that protein turnover was not measured in this observational study. Protein turnover studies, which require intervention with foods labeled with stable isotopes, should be considered in the future to gain insight into the potential impact of protein turnover on circulating concentrations of AGE. Another limitation of the study is it involved the measurement of serum CML and not other circulating AGE. However, circulating AGE show mod-

erate to high correlation in human studies. For example, the Spearman correlation between serum CML and serum imidazolone, another major circulating AGE, was reported as 0.57 ($P < 0.001$) in a study of over 200 patients (36).

The present study provides new epidemiological evidence that fat mass may play a role in the metabolism of AGE. As noted previously, in adipocytes, CD36, a member of the class B scavenger receptor family, serves as a receptor for AGE (13). In vitro studies show that in both murine and human adipocytes, binding of AGE with CD36 is followed by endocytosis and degradation with adipocytes (14). Whether CD36-mediated phagocytosis is simply a physiological mechanism to clear AGE from the circulation is not clear (14). Ligand interaction of AGE with CD36 led to downregulation of leptin expression by adipocytes in vitro (37). Other studies have shown that oxidatively modified proteins accumulate in adipocytes that are exposed to AGE in vitro (38,39). Further insight into the potential effects of body fat on serum CML concentrations may be provided in the future in immunohistochemical studies that involve fat biopsies, which were not possible in the present observational study.

In conclusion, fat mass is inversely associated with serum CML concentrations in community-dwelling adults. Future studies are needed to determine whether serum CML concentrations can be used as a concentration biomarker for dietary intake of AGE.

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