Advanced Glycation End Products and esRAGE Are Associated With Bone Turnover and Incidence of Hip Fracture in Older Men

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Background: Diabetes mellitus is associated with increased fracture risk despite preservation of bone density and reduced bone turnover.

Aims: We tested the hypothesis that circulating advanced glycation end products (AGEs) and endogenous secretory receptor for AGEs (esRAGE) differentially modulate bone turnover and predict fracture risk in older men.

Participants: A total of 3384 community-dwelling men aged 70 to 89 years.

Methods: Collagen type I C-terminal cross-linked telopeptide, *N*-terminal propeptide of type I collagen (P1NP), and total osteocalcin (TOC) were assayed using immunoassay and undercarboxylated osteocalcin (ucOC) following hydroxyapatite binding. Plasma *N*-carboxymethyllysine (CML) and esRAGE were assayed using immunoassay. Methylglyoxal and glyoxal were assayed using mass spectrometry. Incident hip fractures were ascertained.

Results: Median age was 76.3 years (interquartile range, 74.2 to 79.1 years). Plasma CML was measured in 3011 men, methylglyoxal and glyoxal in 766 men, and esRAGE in 748 men. Plasma CML, methylglyoxal, glyoxal, and esRAGE were similar in men without and with diabetes (all $P > 0.05$). CML was positively associated with fasting glucose ($r = 0.06$, $P < 0.001$), and esRAGE was inversely associated ($r = -0.08$, $P = 0.045$). esRAGE was positively associated with bone formation (P1NP, $r = 0.17$, $P < 0.001$; ucOC, $r = 0.11$, $P = 0.008$; TOC, $r = 0.16$, $P < 0.001$). Incident hip fractures occurred in 106 men during follow-up. Men with CML in the third quartile of values had reduced incidence of hip fracture compared with men in the lowest quartile (hazard ratio, 0.49; 95% CI, 0.24 to 0.99; $P = 0.045$).

Conclusions: Glycemia associates positively with CML and reciprocally with esRAGE in older men. Circulating esRAGE modulates bone turnover in older men, whereas CML predicts incidence of hip fracture. (***J Clin Endocrinol Metab*** **103: 4224–4231, 2018)**

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Abbreviations: AGE, advanced glycation end product; BMD, bone mineral density; BMI, body mass index; CML, carboxymethyllysine; CV, coefficient of variation; esRAGE, endogenous secretory receptor for advanced glycation end products; HbA_{1c}, hemoglobin A_{1c}; P1NP, *N*-terminal propeptide of type I collagen; RAGE, receptor for advanced glycation end products; TOC, total osteocalcin; ucOC, undercarboxylated osteocalcin; W2, wave 2; WADLS, Western Australian Data Linkage System.

Diabetes mellitus is associated with increased fracture risk despite preservation of bone mineral density and reduced bone turnover (1–3). Proposed mechanisms include a shift of mesenchymal stem cell differentiation toward adipocytes rather than osteoblasts (4), impaired mineralization due to altered osteoclast differentiation (5–7), osteoblast and osteoclast dysfunction from reduced activity of the insulin receptor substrate (8, 9), elevated sclerostin levels (10), reduced circulating IGF-1 concentrations (10, 11), the effect of chronic inflammation, and the pathogenic effect of advanced glycation end products (AGEs) on bone (1, 2, 12). AGEs are nonenzymatic chemical modifications of proteins by aldose sugars (1, 2, 13, 14) that are associated with macrovascular and microvascular complications of diabetes (13–18). AGEs generate oxidative stress and interact with the receptor for advanced glycation end products (RAGE) (13, 14, 16–18), a transmembrane receptor that activates cell signaling pathways such as the proinflammatory nuclear factor κ-β (14, 17–19).

Of the many AGEs detected in tissue, pentosidine and carboxymethyllysine (CML) are the most prominent and best characterized (2, 13, 18). Reactive intermediate products, including methylglyoxal and glyoxal, are formed during glycation (13, 18), but there have been few studies examining the significance of these compounds. Endogenous secretory RAGE (esRAGE) is an isoform of RAGE that lacks the transmembrane and signaling domain and is believed to act as a decoy receptor, binding AGEs and reducing the AGE-RAGE interaction and activity of related intracellular signaling pathways (20). Previous studies have reported variable associations between diabetes, AGEs, bone turnover, and fracture risk in humans. Bone turnover markers have been reported as unrelated to, or positively associated with, serum pentosidine (21, 22) and negatively associated with CML (23). There is evidence of a positive association between serum pentosidine and fracture risk in cohorts of people with type 1 and type 2 diabetes (21, 22, 24, 25), and a study in the general population demonstrated a positive linear association between CML and hip fracture risk in a cohort of older men and women that was attenuated following adjustment for covariates (26). Low esRAGE levels have been shown to be a risk factor for vertebral fractures in patients with type 2 diabetes (27), and higher esRAGE to pentosidine ratios have been associated with a decreased risk of fragility fractures independent of bone mineral density (BMD) (25).

Osteoporosis is increasingly recognized as a major contributor to morbidity in older men who are also at increased risk of diabetes (28). The relationship between diabetic advanced glycation with both bone physiology and fracture risk remains unclear, particularly in the general population of older men who are at risk for osteoporosis. In this study, we examine the associations between CML, methylglyoxal, glyoxal, and esRAGE with diabetes, bone turnover, and hip fracture risk in a population of older men. We hypothesized that, in older men with or without diabetes, circulating AGEs and esRAGE are associated with bone turnover and fracture risk.

Subjects and Methods

Study population

The Health in Men Study is a cohort of community-dwelling men from Perth, Western Australia (29). Men aged ≥65 years were randomly selected from the electoral roll and invited to participate in the study. There were 12,203 who participated in wave 1 from 1996 to 1999, and 4248 participated in wave 2 (W2) from 2001 to 2004, which comprised reassessment, including repeat blood sampling. Participants were predominantly white. The University of Western Australia Human Research Ethics Committee approved the study, and all men gave written informed consent.

Assessment of medical comorbidities

Medical data collected at W2 were used to identify men with a history of prostate cancer, osteoporosis or bone fracture, and Paget disease. Medication data were analyzed to identify men receiving androgens or antiandrogen therapy, bisphosphonates, or glucocorticoids. Men diagnosed with diabetes, reporting the use of blood glucose–lowering medication, or with fasting or nonfasting blood glucose at W2 of ≥7.0 mmol/L or ≥11.1 mmol/L, respectively, were considered to have diabetes (30, 31).

Ascertainment of incident hip fracture

Further assessment of morbidity was performed via the Western Australian Data Linkage System (WADLS), which provides electronic linkage to records from death, hospital, and cancer registries and captures admissions to all public and private hospitals in Western Australia (32). The WADLS was used to identify admissions to the hospital with falls [International Statistical Classification of Diseases and Related Health Problems, 9th revision (ICD-9): E880 to E886, E888; or ICD-10-Australian Modification (AM): W00 to W19] and hip fracture [ICD-9: 820 (after excluding pathological fractures with 733.1 and 198.5) or ICD-10: S72.0 to S72.2 (after excluding M84.4 and M90.7)]. WADLS data for hip fracture were available from the time of assessment at W2 until the end of 2012.

Laboratory assays

Blood samples were collected between 0800 and 1030 hours at W2. Aliquots of plasma and serum were prepared immediately following phlebotomy and stored at −80°C until assayed. Bone turnover markers, including collagen type 1 C-terminal cross-linked telopeptide, *N*-terminal propeptide of type 1 collagen (P1NP), undercarboxylated osteocalcin (ucOC), and total osteocalcin (TOC), were assayed using immunoassay as previously reported (30, 33). The interassay coefficients of variation (CVs) for the bone turnover markers were 3.7% and 2.9% at 18 and 89 μg/L TOC, 4.0% and 5.7% at 28 and 191 μg/L P1NP, and 4.1% and 3.8% at 0.31 and 0.71 μg/L C-terminal cross-linked telopeptide, respectively. Plasma CML was measured in 3011 men, and methylglyoxal, glyoxal, and esRAGE were measured in a random subset of these men. CML was assayed using a validated indirect ELISA (25).

Interassay CV for CML was 7.9%. esRAGE was assayed using a commercial ELISA (Quantikine; R&D Systems). Interassay CV for esRAGE was 8.2%. Glyoxal and methylglyoxal were determined as published previously (34). In detail, 20 μL of 55% trichloroacetic acid was added to 180 μL of plasma. The sample was vortexed and incubated at room temperature for 20 minutes to allow for protein precipitation to occur and glyoxal and methylglyoxal to be released. The sample was centrifuged at room temperature for 20 minutes at 15,000 rpm in an Eppendorf mini centrifuge. Following centrifugation, 150 μL of the supernatant was transferred into a labeled thermoresistant plastic vial containing 70 μL of 2.5 mM sodium phosphate buffer (pH 9.5) and 35 μL of 5 mM diethylenetriaminepentaacetic acid. Finally, 5 μL of a 3-mM 5,6-diamino-2,4-hydroxypyrimidine sulfate solution was added and the sample incubated at 60°C for 2 hours. After incubation, 14.5 μL of concentrated HCl (37%) was added to adjust the pH to 4.0. Then, 50 μL of the sample was injected into the chromatographic system. A Dionex™ HPLC system (Thermo Fisher Scientific) equipped with a P680 HPLC pump, an ASI-100 automated sample injector, a column oven (thermostated column compartment TCC-100) equipped with a phenyl-hexyl column at 40°C, a PDA ultraviolet/visible detector, and an RF-2000 fluorescent detector were used. The Chromeleon 6.7 Chromatography Data System from Dionex™ (Thermo Fisher Scientific) was used to control the instrument (pump, column oven, and detectors), acquire data, and quantify the peak areas. A composition of formic acid (0.01%)/water (solvent A) and methanol (solvent B) with gradient elution system at a flow rate of 0.75 mL/min was used as a mobile phase. Lumazine (the reaction product of glyoxal) and methyllumazine (the reaction product of methylglyoxal) were quantitated via fluorescence detection (excitation/emission 330/460 nm) using a standard curve with lumazine and methyllumazine standards. The interassay CV was 1.8% for glyoxal and 0.9% for methylglyoxal.

Statistical analysis

Baseline characteristics are shown as mean and SD or numbers and percentages. Linear regression analyses were used to examine the association of CML, methylglyoxal, glyoxal, and esRAGE with fasting glucose and bone turnover markers. As the distribution of CML was skewed to the right, analyses were also performed using ln(CML). Cox proportional hazards regression was performed to assess the association of CML with incident hip fracture. Models were adjusted for variables that might possibly confound the results, including age, smoking, hypertension, dyslipidemia, prevalent cardiovascular disease, prevalent diabetes, frailty, body mass index (BMI)/waist, creatinine, and vitamin D. A two-tailed P value <0.05 was considered significant.

Results

Participant characteristics

Demographic and clinical characteristics are summarized in Table 1. Excluding 24 men with Paget disease of the bone, 127 with known osteoporosis, 192 with previous bone fracture, 11 receiving bisphosphonates, 60 receiving glucocorticoids, and 212 on warfarin, there were 3384 men with data available for analysis. Their mean age was 76.8 years, and their mean BMI was 26.5 kg/m^2. The 502 men with diabetes were more likely to have hypertension, frailty, and cardiovascular disease than the 2882 men without diabetes. The men with diabetes also had a higher BMI and waist circumference, higher serum creatinine, a lower serum vitamin D, and lower bone turnover. Plasma CML was measured in 3011 men, methylglyoxal and glyoxal were measured in 766 men, and esRAGE was measured in 748 men. Plasma CML, methylglyoxal, glyoxal, and esRAGE were similar in men without and with diabetes. When men with diabetes from our study cohort were stratified according to duration of diabetes and use of insulin, there were no significant differences in the distributions of circulating AGEs and esRAGE for men with diabetes duration <5 years and ≥ 5 years or for those on oral therapy and those on insulin (35).

CML, esRAGE, and fasting serum glucose

Associations between AGEs, esRAGE, and fasting serum glucose are summarized in Table 2. In the 2676 men who were fasting at the time of blood sampling and after adjusting for potential confounders, plasma CML was positively associated with fasting serum glucose (fully adjusted analysis: 0.06-SD increase in CML per 1-mmol/L increase in fasting serum glucose, $P < 0.001$). A similar result was seen with ln(CML). esRAGE was inversely associated with fasting serum glucose (fully adjusted analysis: 0.08-SD decrease in esRAGE per 1-mmol/L increase in fasting serum glucose, $P = 0.045$).

esRAGE and bone turnover markers

Associations between AGEs, esRAGE, and bone turnover markers are summarized in Tables 3 through 5. In the multivariable analysis, esRAGE was independently associated with bone turnover, particularly bone formation (per 1-SD increase esRAGE: 0.17-SD increase in P1NP, $P < 0.001$; 0.11-SD increase in ucOC, $P = 0.008$; 0.16-SD increase in TOC, $P < 0.001$) (Table 3). When analyzed according diabetes status, the association between esRAGE and bone turnover markers was more apparent in men with diabetes compared with men without (per 1-SD increase in esRAGE: 0.12-SD increase in P1NP in men without diabetes, $P = 0.017$ and 0.36-SD increase in P1NP in men with diabetes, $P = 0.007$; 0.08-SD increase in TOC in men without diabetes, $P = 0.170$ and 0.24-SD increase in men with diabetes, $P = 0.012$) (Tables 4 and 5).

CML and hip fracture risk

In the 3011 men with CML concentrations, 106 incident hip fractures occurred during follow-up. The cumulative risk of incident hip fracture is shown in Fig. 1, stratified according to quartiles of CML. Men with CML in the third quartile had the lowest cumulative risk. Cox proportional hazards regression models are shown in Table 6. In the fully adjusted analysis, compared with

Table 1. Baseline Characteristics and Distributions of Biochemical Variables, Circulating AGEs, esRAGE, and Bone Turnover Markers in Older Men Without and With Diabetes

	Study Population		Men Without Diabetes		Men With Diabetes		
Variable	**n**	**Value**	**n**	**Value**	**n**	**Value**	***P* Value**
Age, y	3384	76.8 ± 3.5	2882	76.9 ± 3.5	502	76.7 ± 3.4	0.175
Never smoker		1181 (34.9)		1031 (35.8)		150 (29.9)	
Past smoker		2047 (60.5)		1715 (59.5)		332 (66.1)	0.02
Current smoker		156 (4.6)		136 (4.7)		20 (4.0)	
Hypertension		2518 (74.4)		2109 (73.2)		409 (81.5)	<0.001
Dyslipidemia		2465 (72.8)		2082 (72.2)		383 (76.3)	0.06
Frailty		445 (13.2)		331 (11.5)		114 (22.9)	<0.001
Cardiovascular disease		838 (24.8)		647 (22.4)		191 (38.0)	<0.001
BMI, kg/m^2	3374	26.5 ± 3.5	2877	26.3 ± 3.4	497	27.7 ± 3.8	<0.001
Waist, cm	3377	99.0 ± 9.8	2879	98.3 ± 9.6	498	103.1 ± 10.4	<0.001
Fasting glucose, mmol/L	2676	5.6 ± 1.0	2316	5.4 ± 0.5	360	7.3 ± 1.8	<0.001
Creatinine, μmol/L	3384	93.2 ± 31.7	2882	92.7 ± 31.4	502	96.5 ± 33.2	0.012
Vitamin D, nmol/L	3366	68.3 ± 23.2	2865	69.2 ± 23.0	501	63.1 ± 23.8	<0.001
CML, nmol/mol	3011	1482 ± 1665	2566	1463 ± 1467	445	1590 ± 2519	0.137
ln(CML)	3011	6.7 ± 1.5	2566	6.7 ± 1.5	445	6.7 ± 1.5	0.537
Glyoxal, nmol/L	766	717 ± 334	650	723 ± 337	116	688 ± 318	0.31
Methylglyoxal, nmol/L	766	530 ± 201	650	528 ± 198	116	539 ± 216	0.583
esRAGE, pg/mL	748	1625 ± 848	634	1648 ± 833	114	1501 ± 920	0.088
CTX, ng/mL	3383	0.3 ± 0.2	2882	0.3 ± 0.2	501	0.3 ± 0.2	<0.001
P1NP, ng/mL	3383	43.0 ± 28.7	2881	43.8 ± 28.5	502	38.4 ± 29.4	<0.001
ucOC, ng/mL	3382	11.1 ± 5.3	2880	11.4 ± 5.0	502	9.7 ± 6.4	<0.001
TOC, ng/mL	3384	21.1 ± 12.8	2882	21.6 ± 11.3	502	18.7 ± 18.9	<0.001

Variables of interest: n = number of men from the study population after exclusions, in whom that result is available. Men diagnosed with diabetes, reporting use of glucose-lowering medication, or with fasting glucose at W2 of ≥7 mmol/L or nonfasting ≥11.1 mmol/L were considered to have diabetes. Data are shown as mean ± SD or n (%). *P* value is for comparison between men without and with diabetes.

Abbreviation: CTX, C-terminal cross-linked telopeptide.

men with CML in the lowest (reference) quartile, men in the third quartile had a lower incidence of hip fracture (hazard ratio, 0.49; 95% CI, 0.24 to 0.99; $P = 0.045$).

Discussion

This study demonstrates that, in older men, fasting serum glucose correlated with serum CML and inversely with the decoy receptor esRAGE. esRAGE was independently associated with markers of bone formation. Furthermore, there was evidence of a nonlinear association between CML and incidence of hip fracture.

The positive association between fasting serum glucose and CML is consistent with previous evidence that chronic hyperglycemia accelerates the process of nonenzymatic glycation (12, 18). Several studies have examined the relationship between glycemia and AGEs with varying results. Makita *et al.* (36) demonstrated elevated AGEs in arterial wall collagen samples obtained at autopsy and in the circulation of living subjects with diabetes compared with those without diabetes. Miura *et al.* (15) found no correlation between serum AGEs and fasting plasma glucose but did report a significant correlation between both serum levels of CML

Table 2. Associations of AGEs and esRAGE With Fasting Glucose in Older Men

	SD Change per 1-mmol/L Increase in Fasting Glucose (95% CI) and *P* Value	
Variable	**Unadjusted**	**Fully adjusted**
CML	0.09 (0.04 to 0.13) <0.001	0.06 (0.03 to 0.09) < 0.001
ln(CML)	0.04 (0.00 to 0.08) 0.045	0.04 (0.00 to 0.07) 0.027
Glyoxal	−0.01 (−0.09 to 0.08) 0.867	−0.00 (−0.07 to 0.06) 0.908
Methylglyoxal	−0.06 (−0.16 to 0.04) 0.223	−0.04 (−0.11 to 0.04) 0.351
esRAGE	−0.13 (−0.21 to −0.04) 0.004	−0.08 (−0.15 to −0.00) 0.045

Standardized regression coefficients for AGEs and esRAGE with fasting glucose in 2676 older men who were fasting at time of blood sampling, adjusted for age, smoking, hypertension, dyslipidemia, prevalent cardiovascular disease, prevalent diabetes, frailty, BMI/waist, creatinine, and vitamin D. Data are shown as fold-SD change in AGEs or esRAGE per 1-unit increase in fasting glucose (95% CI) and *P* value.

Table 3. Standardized Regression Coefficients for AGEs and esRAGE With Serum Markers of Bone Turnover in Older Men

Variable	CTX	P1NP	ucOC	TOC
CML	−0.01 (−0.05 to 0.03) 0.515	−0.01 (−0.05 to 0.03) 0.657	0.00 (−0.04 to 0.05) 0.849	0.00 (−0.04 to 0.05) 0.962
ln(CML)	−0.03 (−0.07 to 0.01) 0.141	−0.01 (−0.05 to 0.02) 0.475	0.01 (−0.03 to 0.05) 0.677	0.00 (−0.04 to 0.05) 0.853
Glyoxal	−0.09 (−0.19 to 0.01) 0.064	−0.07 (−0.16 to 0.02) 0.106	−0.06 (−0.15 to 0.02) 0.158	−0.04 (−0.14 to 0.06) 0.403
Methylglyoxal	−0.00 (−0.09 to 0.08) 0.976	0.00 (−0.07 to 0.08) 0.946	−0.01 (−0.08 to 0.07) 0.863	0.03 (−0.05 to 0.11) 0.469
EsRAGE	0.06 (−0.03 to 0.14) 0.175	0.17 (0.09 to 0.26) <0.001	0.11 (0.03 to 0.18) 0.008	0.16 (0.07 to 0.24) <0.001

Adjusted for age, smoking, hypertension, dyslipidemia, prevalent cardiovascular disease, prevalent diabetes, frailty, BMI/waist, creatinine, vitamin D, and fasting glucose. Data are shown as fold-SD increase in bone turnover marker per 1-SD increase in AGEs and esRAGE (95% CI) and *P* value.

Abbreviation: CTX, C-terminal cross-linked telopeptide.

and non-CML AGE and hemoglobin A_{1c} (HbA_{1c}) in persons with type 1 diabetes. Serum pentosidine levels did not correlate with HbA_{1c} (15). A study by Neumann *et al.* (24) also demonstrated no correlation between serum pentosidine with HbA_{1c} in persons with type 1 diabetes, but there was a significant correlation between serum pentosidine and diabetes duration. In a cohort study of older men and women, Schwartz *et al.* (21) showed that serum pentosidine did not correlate with HbA_{1c} and that pentosidine concentrations were similar in persons with and without diabetes.

We found no significant difference in the concentrations of AGEs and esRAGE in older men with diabetes compared with those without diabetes. Serum CML levels were higher and esRAGE was lower in men with diabetes compared with other men, but these differences were not significant, perhaps reflecting the large interindividual variation resulting in overlapping distributions of these analytes in the two groups. By contrast, serum CML was positively associated with fasting glucose, consistent with mediation of circulating AGEs by glycemia. Neither of the intermediates glyoxal or methylglyoxal was associated with fasting serum glucose. The robust association we demonstrated between serum CML and fasting glucose may be accounted for by our large cohort of men predominantly without diabetes and therefore with stable fasting glucose levels that were not influenced by treatments such as insulin. The negative association between esRAGE and fasting serum glucose would be consistent with hyperglycemia suppressing esRAGE. Given the proposed role of esRAGE as a decoy receptor acting to reduce the pathogenic effects of the AGE-RAGE interaction (20), a lower circulating esRAGE concentration in the setting of hyperglycemia would be expected to increase the deleterious effects of AGEs.

AGEs have multiple biological effects on bone metabolism that should result in increased bone fragility. *In vitro* studies have demonstrated that they affect multiple cell lines with inhibition of osteoblast proliferation and induction of osteoblast apoptosis (37, 38), as well as decreasing osteoclast-induced bone resorption and osteoclast differentiation (39). AGEs have been shown to increase the expression of sclerostin and decrease the expression of receptor activator of nuclear factor kappa-B ligand in osteocytelike cells (40). Overall, these effects suggest that the pathogenesis of reduced bone turnover in diabetes may be due to accumulation of AGEs (2). Furthermore, AGEs alter the biomechanical properties of bone tissue via nonenzymatic cross-linking of collagen, resulting in impaired bone quality and increased fragility (3, 41).

We demonstrated that esRAGE is positively correlated with the bone formation markers P1NP, ucOC, and TOC with a stronger association in men with diabetes compared with men without diabetes. Conversely, lower circulating esRAGE was associated with reduced bone formation. We postulate that lower circulating esRAGE may

Table 4. Standardized Regression Coefficients for AGEs and esRAGE With Serum Markers of Bone Turnover in Older Men Without Diabetes

Variable	CTX	P1NP	ucOC	TOC
CML	−0.01 (−0.04 to 0.03) 0.657	−0.01 (−0.04 to 0.03) 0.717	0.00 (−0.03 to 0.04) 0.816	0.00 (−0.04 to 0.05) 0.826
ln(CML)	−0.03 (−0.08 to 0.01) 0.146	−0.02 (−0.06 to 0.02) 0.348	0.00 (−0.04 to 0.05) 0.909	−0.00 (−0.06 to 0.05) 0.922
Glyoxal	−0.08 (−0.19 to 0.02) 0.108	−0.09 (−0.19 to 0.01) 0.070	−0.09 (−0.19 to 0.01) 0.088	−0.07 (−0.20 to 0.05) 0.248
Methylglyoxal	0.02 (−0.07 to 0.11) 0.670	0.01 (−0.07 to 0.10) 0.742	0.00 (−0.09 to 0.09) 0.976	0.07 (−0.04 to 0.18) 0.210
EsRAGE	0.04 (−0.05 to 0.13) 0.333	0.12 (0.02 to 0.22) 0.017	0.04 (−0.05 to 0.14) 0.350	0.08 (−0.03 to 0.20) 0.170

Adjusted for age, smoking, hypertension, dyslipidemia, prevalent cardiovascular disease, prevalent diabetes, frailty, BMI/waist, creatinine, vitamin D, and fasting glucose. Data are shown as fold-SD increase in bone turnover marker per 1-SD increase in AGEs and esRAGE (95% CI) and *P* value.

Abbreviation: CTX, C-terminal cross-linked telopeptide.

Table 5. Standardized Regression Coefficients for AGEs and esRAGE With Serum Markers of Bone Turnover in Older Men With Diabetes

Variable	CTX	P1NP	ucOC	TOC
CML	−0.10 (−0.32 to 0.12) 0.370	−0.10 (−0.31 to 0.11) 0.352	−0.07 (−0.28 to 0.14) 0.530	−0.09 (−0.30 to 0.11) 0.359
ln(CML)	−0.03 (−0.16 to 0.09) 0.597	0.01 (−0.12 to 0.13) 0.933	0.03 (−0.09 to 0.15) 0.625	0.02 (−0.10 to 0.13) 0.785
Glyoxal	−0.16 (−0.47 to 0.16) 0.325	0.02 (−0.32 to 0.37) 0.893	0.04 (−0.24 to 0.31) 0.787	0.03 (−0.22 to 0.27) 0.836
Methylglyoxal	−0.18 (−0.45 to 0.09) 0.197	−0.01 (−0.31 to 0.29) 0.939	−0.01 (−0.26 to 0.23) 0.912	−0.01 (−0.22 to 0.20) 0.927
EsRAGE	0.13 (−0.10 to 0.36) 0.265	0.36 (0.10 to 0.62) 0.007	0.20 (−0.01 to 0.40) 0.061	0.24 (0.06 to 0.43) 0.012

Adjusted for age, smoking, hypertension, dyslipidemia, prevalent cardiovascular disease, prevalent diabetes, frailty, BMI/waist, creatinine, vitamin D, and fasting glucose. Data are shown as fold-SD increase in bone turnover marker per 1-SD increase in AGEs and esRAGE (95% CI) and *P* value.

Abbreviation: CTX, C-terminal cross-linked telopeptide.

predispose men to greater deleterious effects of AGEs in bone, acting via RAGE in the absence of the decoy receptor. If circulating esRAGE is reduced in the setting of fasting hyperglycemia, this may increase bone fragility and fracture risk.

We found no correlations between CML and the different bone turnover markers. Other assessing associations between AGEs and bone turnover markers have reported inconsistent results. Schwartz *et al.* (21) demonstrated that markers of bone formation and resorption had small but significant positive associations with pentosidine levels. However, Yamamoto *et al.* (22) found no correlation between serum pentosidine and bone metabolic markers. Further research is needed to clarify the relationship between AGEs such as CML and bone turnover.

We found evidence of a nonlinear association between CML and incident hip fracture in older men. Men with CML in the third quartile of values had approximately half the risk of hip fracture compared with men with CML in the lowest quartile. The association between AGEs and fracture risk has been studied previously in different subject groups. Neumann *et al.* (24) demonstrated that high serum pentosidine was associated with prevalent fractures in type 1 diabetes independent of bone mineral density. Schwartz *et al.* (21) found that pentosidine was associated with fracture risk in older adults with diabetes but not in those without diabetes. Yamamoto *et al.* (22) demonstrated that serum pentosidine levels were positively associated with vertebral fractures in postmenopausal women with type 2 diabetes, also independent of BMD. Tamaki *et al.* (25) have shown in a population of older men that increased esRAGE to pentosidine ratio levels were associated with a decreased fracture risk independent of BMD and HbA_{1c}. Barzilay *et al.* (26) reported that CML levels were associated with hip fracture risk in older adults independent of BMD and with no difference between patients with and without diabetes. Our findings differ from these studies in identifying a nonlinear or U-shaped association between circulating CML and hip fracture risk. This association was robust after adjustment for potential confounders with a substantial effect size demonstrated by the hazard ratio of 0.49 for men with CML in the third quartile. Our findings are consistent with evidence that the pathogenic effects of AGEs on bone are multifactorial and may cause biomechanical changes, deteriorating bone strength via nonenzymatic cross-linking of collagen without causing cellular dysfunction (3, 12, 41). Thus, is it possible that AGEs such as CML may increase bone fragility and fracture risk without altering bone turnover. Our findings support the potential utility of CML as a biomarker for risk of incident hip fracture in older men. However, further studies are needed to clarify why this association is U-shaped rather than linear and determine the optimal range of CML that is associated with the lowest risk of fracture.

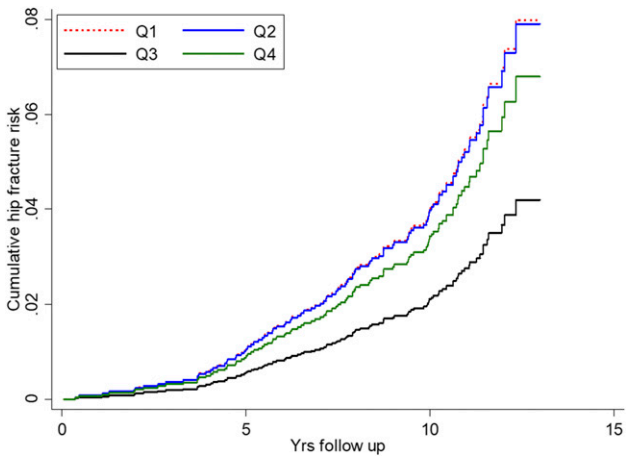

Figure 1. Nelson-Aalen plot showing cumulative risk of incident hip fracture in 3011 community-dwelling older men, stratified according to quartiles of CML. Q, quartile.

The strengths of this study are that we studied a large population of older men with parallel assays of CML, methylglyoxal, glyoxal, and esRAGE to assess the entire pathway of AGEs. We assessed the association with a panel of bone turnover markers and hip fracture risk. Multivariable regression analyses were conducted to assess associations of AGEs and esRAGE with fasting glucose and bone turnover markers, as well as CML with hip fracture, adjusting for age, smoking, hypertension, dyslipidemia, prevalent cardiovascular disease, prevalent diabetes, frailty, BMI/waist, creatinine, and vitamin D. This study has several limitations. We analyzed a single

Table 6. Association of CML With Incident Hip Fracture in Older Men

CML	Incident Hip Fracture, n	Hazard Ratio (95% CI) and *P* Value	
		Unadjusted	Fully Adjusted
Q1 (reference)	31	1.00	1.00
Q2	30	0.98 (0.60−1.63) 0.951	1.13 (0.64−2.00) 0.680
Q3	17	0.53 (0.29−0.95) 0.033	0.49 (0.24−0.99) 0.045
Q4	28	0.85 (0.51−1.42) 0.545	0.70 (0.37−1.33) 0.279

Cox proportional hazards regression with the outcome of incident hip fracture according to CML in quartiles. Data are hazard ratios (95% CIs) and *P* values, unadjusted and adjusted for age, smoking, hypertension, dyslipidemia, prevalent cardiovascular disease, prevalent diabetes, frailty, BMI/waist, creatinine, vitamin D, and fasting glucose.

Abbreviation: Q, quartile.

blood sample at baseline and did not have serial data, and only a random subset of participants had assays for glyoxal, methylglyoxal, and esRAGE. Although no associations were seen for glyoxal and methylglyoxal, the results for esRAGE were informative. Analyses were adjusted for age, and thus the associations are less likely to represent confounding from this source. However, the cohort comprises older men, and the results may not apply to younger men. HbA_{1c} was not analyzed in this cohort. We analyzed fasting glucose levels, which may be a less accurate marker of glycemic control and severity of disease. WADLS captures data for incident hip fractures, as men with hip fractures almost invariably present to the hospital. We did not analyze vertebral fractures as WADLS does not comprehensively capture these events given that a proportion of men with vertebral fractures are managed without hospitalization. Nevertheless, hip fractures are the major cause for morbidity in older men. As this was an observational study, we are unable to prove causation. Finally, we cannot make any comment on associations in women.

In conclusion, there appears to be a complex interaction between glycemia, AGEs, esRAGE, and bone such that AGEs and esRAGE are associated with hyperglycemia in reciprocal fashion, esRAGE with bone formation, and CML with fracture risk in older men. Further studies are needed to assess whether CML could be used to improve risk stratification for hip fracture in older men and to test whether improving glycemic control in men with diabetes would modulate AGEs and esRAGE and possibly reduce fracture risk.

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