Published in final edited form as:

Nutrition. 2012 July ; 28(7-8): 762–766. doi:10.1016/j.nut.2011.11.005.

Circulating selenium and carboxymethyl-lysine, an advanced glycation end product, are independent predictors of anemia in older community-dwelling adults

Cindy N. Roy, Ph.D.^a, Richard D. Semba, M.D., M.P.H.^b, Kai Sun, M.S.^b, Stefania Bandinelli, M.D.^c, Ravi Varadhan, Ph.D.^a, Kushang V. Patel, Ph.D., M.P.H.^d, Jack M. Guralnik, M.D., Ph.D.^d, and Luigi Ferrucci, M.D., Ph.D.^e

^aDivision of Geriatric Medicine and Gerontology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

^bDepartment of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

^cAzienda Sanitaria Firenze, Florence, Italy

^dLaboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, Bethesda, Maryland, USA

^eLongitudinal Studies Section, Clinical Research Branch, National Institute on Aging, Baltimore, Maryland, USA

Abstract

Objective—To assess whether selenium and carboxymethyl-lysine (CML), two biomarkers of oxidative stress, are independent predictors of anemia in older, community-dwelling adults.

Methods—Plasma selenium, CML, folate, vitamin B₁₂, testosterone, and markers of iron status and inflammation were measured at baseline in 1,036 adults, 65 years, in the InCHIANTI Study, a population-based cohort study of aging in Tuscany, Italy, and examined in relationship to prevalent anemia and incident anemia over 6 years of follow-up.

Results—At enrollment, 11.6% of participants were anemic. Of 472 participants who were non-anemic at enrollment 72 (15.3%) developed anemia within 6 years of follow-up. At enrollment, plasma CML in the highest quartile (>425 ng/mL) and plasma selenium in the lowest quartile (<66.6 µg/L) predicted incident anemia (Hazards Ratio [H.R.] 1.67, 95% Confidence Interval [C.I.] 1.07–2.59, *P* = 0.02; H.R. 1.55, 95% C.I. 1.01–2.38, *P* = 0.05, respectively) in a multivariate Cox proportional hazards model that adjusted for age, education, body mass index, cognition, inflammation, red cell distribution width, ferritin, vitamin B₁₂, testosterone, and chronic diseases.

Conclusion—Elevated plasma carboxymethyl-lysine and low plasma selenium are long-term independent predictors of anemia among older community-dwelling adults. These findings support the idea that oxidative stress contributes to the development of anemia.

© 2011 Elsevier Inc. All rights reserved.

Correspondence to: Richard D. Semba, M.D., M.P.H., Johns Hopkins University School of Medicine, Smith Building, M015, 400 N. Broadway, Baltimore, MD 21287; Phone (410) 955-3572, fax (410) 502-1753; rdsemba@jhmi.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

advanced glycation end products; aging; anemia; carboxymethyl-lysine; oxidative stress; selenium

Introduction

Anemia is common in older adults [1] and is associated with a wide spectrum of adverse outcomes, including reduced quality of life [2], decreased mobility [3], increased disability [4], decreased muscle strength [5], depression [6], impaired cognitive function [7], and increased mortality [8]. Anemia is also linked with congestive heart failure [9] and higher risk of Alzheimer's disease [10]. The reduction of oxygen-carrying capacity of the blood that occurs with anemia may account for fatigue, cardiovascular complications, and impaired physical performance, but epidemiologic evidence cannot prove that anemia is the cause of disability and mortality in this population. Clinical trials to test the efficacy of anemia correction for the improvement of physical performance and morbidity in older adults are lacking.

Factors which are usually implicated in the pathogenesis of anemia among older adults include chronic inflammation, renal disease, iron deficiency, and folate and vitamin B₁₂ deficiency [11]. Low testosterone levels are also associated with anemia in both older men and older women [12]. Our recent studies suggest that oxidative stress is a pathophysiological mechanism that may explain anemia in older adults. Selenium is a component of selenoproteins, including selenoenzymes such as glutathione peroxidase, selenoprotein-P, and thioredoxin reductase. Low serum selenium concentrations are associated with anemia in US adults [13]. Elevated serum carboxymethyl-lysine (CML), a dominant advanced glycation end product (AGE) and circulating receptors for AGEs [14] are associated with anemia. Humans are exposed to CML through endogenous generation of CML by abnormal glucose metabolism and by lipoxidation, and through exogenous CML found in foods. AGEs are known to reduce the deformability of erythrocytes and could potentially decrease erythrocyte lifespan.

The prevalence and correlates of anemia in older adults have been examined mostly in cross-sectional studies. A study from Olmsted County, Minnesota [15] based upon hemoglobin determinations obtained for clinical use in 618 men and women aged 65 years and older estimated annual incidence rates of anemia of 90.3/1000 in men and 69.1/1000 in women. About three-fourths of cases of anemia were detected in relation to a hospitalization, and blood loss accounted for about half the cases of anemia. Whether biomarkers of oxidative stress, such as selenium and CML, or other biomarkers are long-term predictors of anemia in older adults is not clear [1]. If such biomarkers exist, their discovery is important because they may be useful for screening older individuals at high risk of developing anemia, and may also suggest clues to the pathogenesis of anemia in older persons.

Using data from a large, population-based study of community-dwelling older adults, the aim of our study was to identify circulating biomarkers that are risk factors for incident anemia among older adults.

Subjects and Methods

Study population

Study participants were 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 [16]. Briefly, in August 1998, 1270 people aged 65 years and older were randomly selected from the population registry of Greve in Chianti (pop. 11,709) and Bagno a Ripoli (pop. 4,704), and of 1,256 eligible subjects, 1,155 (90.1%) agreed to participate. Of the 1,155 participants, 1,043 (90.3%) participated in the blood drawing at baseline and 3 and 6 years of follow up. During six years of follow-up, 227 participants died, 34 refused to participate in the study and 16 moved away from the area. 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 secondary data analysis was approved by the John Hopkins School of Medicine Institutional Review Board.

Data collection

Demographic information and information on smoking and medication use were collected using standardized questionnaires [16]. Education was recorded as years of school. 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, chronic heart failure, stroke, and cancer [17]. Fasting plasma glucose was defined as normal, impaired, or diabetic based upon a fasting plasma glucose of ≤ 99 mg/dL, 100–125 mg/dL, and >125 mg/dL, respectively [18]. The diagnosis of diabetes was based upon the diagnostic algorithm [17], and among those who reported no diabetes, by a fasting plasma glucose >125 mg/dL [19]. 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 $\text{weight}/\text{height}^2$ (kg/m^2). Mini-Mental State Examination (MMSE) was administered at enrollment, and an MMSE score <24 was considered consistent with cognitive impairment [20]. Chronic kidney disease 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 [21]. The participants in the study were not taking multivitamins, folate, iron, or vitamin B₁₂ supplements.

Laboratory analyses

Blood samples were collected in the morning after a 12-h fast. Complete blood count was obtained using a hematology autoanalyzer (Sysmex SE 9000, Bucks, United Kingdom) at enrollment, 3-year, and 6-year follow-up visits. Aliquots of serum and plasma were immediately obtained and stored at -80°C . Aliquots of 24-hour urine were stored at -80°C and were not thawed until analysis. Serum and urinary creatinine were measured using a modified Jaffe method and used to calculate creatinine clearance.

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 [22]. CML was measured using a competitive ELISA (AGE-CML ELISA, Microcoat, Penzberg, Germany) [23]. This assay has been validated [24], is specific, and shows no cross-reactivity with other compounds [23]. Selenium was measured using graphite furnace atomic absorption spectrometry [13]. High sensitivity plasma C-reactive protein was measured in duplicate using an immunoturbidimetric assay (Roche Diagnostics) with a minimum detectable concentration of 1 pg/mL. Interleukin-6 (IL-6) was measured in duplicate by high sensitivity enzyme-linked immuno-absorbent assay (ELISA) using a commercial kit (Biosource International, Camarillo, CA) with a minimum detectable concentration of 0.1 pg/mL. Total testosterone was measured using a commercial kit (Diagnostic Systems Laboratories, Webster, TX) with a minimum detection

limit of 0.03 nmol/L. Concentration of bioavailable testosterone was calculated using the Vermeulen formula [25].

Statistical analysis

Variables are reported as medians (25th, 75th percentiles) or as percentages. Characteristics of subjects according to whether or not they had anemia at enrollment were compared using Wilcoxon rank sum tests for continuous variables and chi-square tests for categorical variables. Anemia was defined as hemoglobin concentration <12 g/dL in women and <13 g/dL in men, according to the World Health Organization [26]. Cox proportional hazards models were used to examine the relationship between variables at baseline and incident anemia at the 3- and 6-year follow-up visits. The assumptions of the Cox proportional hazards models were checked using time interaction, and models were checked for goodness-of-fit and for robustness of outliers. The statistical program used was SAS (SAS Institute, Cary, NC). The level of significance used in this study was $P < 0.05$.

Results

Overall, median (25th, 75th percentile) plasma CML concentrations were 350 (289, 425) ng/mL, and plasma selenium concentrations were 74.2 (66.6, 82.3) µg/L. At enrollment, of 1036 participants, 120 (11.6%) were anemic. The characteristics of participants with and without anemia at enrollment are shown in Table 1. Older age and lower education, BMI, MMSE score, ferritin, red cell distribution width (RDW), vitamin B₁₂, total testosterone, bioavailable testosterone, and selenium were associated with anemia. Elevated CML, IL-6, and CRP were associated with anemia. Chronic diseases that were associated with anemia were stroke, depression, and chronic kidney disease. Sex, current smoking, folate, hypertension, angina, heart failure, peripheral artery disease, diabetes mellitus, and cancer were not associated with anemia.

There were 472 participants who were not anemic at enrollment. Within 6 years of follow-up, 72 (15.3%) participants became anemic. Multivariate Cox proportional hazards models were used to characterize the relationship between demographic and other factors at enrollment with incident anemia during follow-up (Table 2). Three models were used to examine the relationship of CML and selenium with incident anemia. Covariates included in the models were variables that were significant in the bivariate analyses shown in Table 1. The first model adjusted for basic demographic factors, the second model added laboratory markers, and the third model adjusted additionally for chronic diseases. Age, CML and selenium were independent predictors of anemia in models that adjusted for age, education, BMI, MMSE score (Model 1). CML was an independent predictor of anemia in Model 2, which additionally adjusted for IL-6, CRP, ferritin, vitamin B₁₂, and testosterone. In Model 3, CML and selenium were independent predictors of anemia after chronic diseases (stroke, depression, and chronic kidney disease) were added to the same covariates as in Model 2. In Model 3, IL-6, ferritin, vitamin B₁₂, testosterone, stroke, depression, and chronic kidney disease were not significant predictors of anemia.

Discussion

The present study showed that older adults with elevated plasma CML or low plasma selenium were at risk for developing anemia over six years of follow-up. The present study corroborated previous cross-sectional studies that showed an association between low serum selenium and anemia in the U.S. National Health and Nutrition Examination Survey III [13] and between elevated serum CML and anemia among older, moderately to severely disabled women living in the community [14]. The present study expanded these observations, and to

our knowledge, is the first study to show that plasma CML and selenium, two markers of oxidative stress, are independent predictors of incident anemia.

AGEs, such as CML, are known to accumulate in erythrocytes over time and alter their deformability [27, 28]. The decreased deformability induced by AGEs can be reversed by AGE inhibitors [28]. In addition, AGEs that accumulate on the surface of erythrocytes can bind with the receptor for AGEs (RAGE) on the vascular endothelium [29]. The binding of AGEs with RAGE [30] may activate the NF- κ B pathway and upregulate inflammatory cytokines such as IL-6 [31]. Although changes in the deformability of erythrocytes induced by AGEs and AGE-RAGE binding are plausible biological explanations for the association between AGEs and anemia, whether a causal relationship exists would need to be addressed by a controlled intervention study or clinical trial.

Selenium is a required co-factor for the antioxidant protein, glutathione peroxidase (Gpx) [32]. Regulation of oxidative stress is an essential requirement for oxygen-carrying erythrocytes, which express Gpx, among other antioxidant enzymes. Patients supported by parenteral nutrition who have low serum selenium concentrations have also been shown to have low red blood cell selenium. Further, selenium supplementation has been shown to increase serum selenium, red blood cell selenium and Gpx activity in a patient receiving parenteral nutrition [33]. Whole blood Gpx activity has been shown to decline with age [34], but whether this is directly related to selenium concentration is, as yet, unclear. More broadly, deficiencies in antioxidant enzymes result in decreased erythroid maturation [35] and shortened erythrocyte lifespan [35, 36]. In mice, antioxidant interventions partially correct this anemia. In aged rats, antioxidant supplementation has been shown to positively impact on end organs, such as muscle [37]. Together, these studies imply that there may be a possible therapeutic effect of selenium or antioxidant supplementation in older adults with low selenium and anemia.

Low antioxidant activity resulting from low selenium concentrations and reduced erythrocyte deformability mediated by AGEs would both be expected to reduce erythrocyte life span in at-risk older adults. Future studies concerning the pathogenesis of anemia in older adults should investigate erythrocyte survival and the efficacy of selenium supplementation or antioxidant administration for the treatment of anemia in this vulnerable population.

Both elevated CML [38] and low selenium [39] have been shown to predict mortality. The relationship between these covariates and incident anemia in this study may be underestimated because of the competing risk of mortality. Some of the subjects may have become anemic before their death, and before hemoglobin was determined at year 3 or 6 of the study. Red cell distribution width (RDW) is thought to reflect increased oxidative stress and inflammation [40], but RDW was not a predictor of anemia in the multivariable models.

This study was somewhat limited by 3 year time intervals for follow up compared with annual follow up, since more frequent study intervals might be expected to detect additional cases of incident anemia during the six-year follow-up period. While the identified risks for development of anemia are strong, they cannot necessarily attribute the cause of anemia in this population to high CML or low selenium. Another limitation is that dietary intake of AGEs were not assessed; however, serum CML has been reported to correlate with dietary intake of AGEs [41].

Conclusion

Plasma CML and selenium, two biomarkers of oxidative stress, are independent predictors of anemia in older community-dwelling adults.

Acknowledgments

The work was supported by National Institute on Aging Grants R01 AG027012, R01 AG029148, the Italian Ministry of Health (ICS110.1/RF97.71), NIA contracts 263 MD 9164, 263 MD 821336, N.1-AG-1-1, N.1-AG-1-2111, and N01-AG-5-0002, and the Intramural Research Program, National Institute on Aging, National Institutes of Health. CNR was additionally supported by the American Society of Hematology, RO1 DK082722 and the Research Career Development Core of the Johns Hopkins Older Americans Independence Center (AG021334). The InCHIANTI study baseline (1998-2000) was supported as a “targeted project” (ICS110.1/RF97.71) by the Italian Ministry of Health and in part by the US National Institute on Aging (contracts 263 MD 9164 and 263 MD 821336).

References

1. Patel KV. Epidemiology of anemia in older adults. *Semin Hematol*. 2008 Oct; 45(4):210–7. [PubMed: 18809090]
2. Valderrabano F. Quality of life benefits of early anaemia treatment. *Nephrol Dial Transplant*. 2000; 15(Suppl 3):23–8. [PubMed: 11032354]
3. Chaves PH, Ashar B, Guralnik JM, Fried LP. Looking at the relationship between hemoglobin concentration and prevalent mobility difficulty in older women. Should the criteria currently used to define anemia in older people be reevaluated? *Journal of the American Geriatrics Society*. 2002 Jul; 50(7):1257–64. [PubMed: 12133021]
4. Penninx BW, Pahor M, Cesari M, Corsi AM, Woodman RC, Bandinelli S, et al. Anemia is associated with disability and decreased physical performance and muscle strength in the elderly. *Journal of the American Geriatrics Society*. 2004 May; 52(5):719–24. [PubMed: 15086651]
5. Cesari M, Penninx BW, Lauretani F, Russo CR, Carter C, Bandinelli S, et al. Hemoglobin levels and skeletal muscle: results from the InCHIANTI study. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*. 2004 Mar; 59(3):249–54.
6. Onder G, Penninx BW, Cesari M, Bandinelli S, Lauretani F, Bartali B, et al. Anemia is associated with depression in older adults: results from the InCHIANTI study. *J Gerontol A Biol Sci Med Sci*. 2005 Sep; 60(9):1168–72. [PubMed: 16183958]
7. Peters R, Burch L, Warner J, Beckett N, Poulter R, Bulpitt C. Haemoglobin, anaemia, dementia and cognitive decline in the elderly, a systematic review. *BMC geriatrics*. 2008; 8:18. [PubMed: 18691409]
8. Patel KV, Longo DL, Ershler WB, Yu B, Semba RD, Ferrucci L, et al. Haemoglobin concentration and the risk of death in older adults: differences by race/ethnicity in the NHANES III follow-up. *British journal of haematology*. 2009 May; 145(4):514–23. [PubMed: 19344387]
9. Silverberg DS, Wexler D, Blum M, Keren G, Sheps D, Leibovitch E, et al. The use of subcutaneous erythropoietin and intravenous iron for the treatment of the anemia of severe, resistant congestive heart failure improves cardiac and renal function and functional cardiac class, and markedly reduces hospitalizations. *Journal of the American College of Cardiology*. 2000 Jun; 35(7):1737–44. [PubMed: 10841219]
10. Beard CM, Kokmen E, O’Brien PC, Ania BJ, Melton LJ 3rd. Risk of Alzheimer’s disease among elderly patients with anemia: population-based investigations in Olmsted County, Minnesota. *Annals of epidemiology*. 1997 Apr; 7(3):219–24. [PubMed: 9141646]
11. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood*. 2004 Oct 15; 104(8):2263–8. [PubMed: 15238427]
12. Ferrucci L, Maggio M, Bandinelli S, Basaria S, Lauretani F, Ble A, et al. Low testosterone levels and the risk of anemia in older men and women. *Archives of internal medicine*. 2006 Jul 10; 166(13):1380–8. [PubMed: 16832003]
13. Semba RD, Ricks MO, Ferrucci L, Xue QL, Guralnik JM, Fried LP. Low serum selenium is associated with anemia among older adults in the United States. *European journal of clinical nutrition*. 2009 Jan; 63(1):93–9. [PubMed: 17805227]
14. Semba RD, Ferrucci L, Sun K, Patel KV, Guralnik JM, Fried LP. Elevated serum advanced glycation end products and their circulating receptors are associated with anaemia in older community-dwelling women. *Age and ageing*. 2009 May; 38(3):283–9. [PubMed: 19252206]

15. Ania BJ, Suman VJ, Fairbanks VF, Rademacher DM, Melton LJ 3rd. Incidence of anemia in older people: an epidemiologic study in a well defined population. *J Am Geriatr Soc.* 1997 Jul; 45(7): 825–31. [PubMed: 9215333]
16. Ferrucci L, Bandinelli S, Benvenuti E, Di Iorio A, Macchi C, Harris TB, et al. Subsystems contributing to the decline in ability to walk: bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. *J Am Geriatr Soc.* 2000 Dec; 48(12):1618–25. [PubMed: 11129752]
17. Guralnik, JMFL.; Simonsick, EM., et al. The Women's Health and Aging Study: Health and Social Characteristics of Older Women with Disability. Bethesda, MD: National Institute on Aging; NIH Publication. 1995;95–4009
18. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes care.* 2003 Jan; 26(Suppl 1):S5–20. [PubMed: 12502614]
19. Diagnosis and classification of diabetes mellitus. *Diabetes care.* 2006 Jan; 29(Suppl 1):S43–8. [PubMed: 16373932]
20. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research.* 1975 Nov; 12(3):189–98. [PubMed: 1202204]
21. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. Modification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Annals of internal medicine.* 1999 Mar 16; 130(6):461–70. [PubMed: 10075613]
22. Reddy S, Bichler J, Wells-Knecht KJ, Thorpe SR, Baynes JW. N epsilon-(carboxymethyl)lysine is a dominant advanced glycation end product (AGE) antigen in tissue proteins. *Biochemistry.* 1995 Aug 29; 34(34):10872–8. [PubMed: 7662668]
23. Boehm BO, Schilling S, Rosinger S, Lang GE, Lang GK, Kientsch-Engel R, et al. Elevated serum levels of N(epsilon)-carboxymethyl-lysine, an advanced glycation end product, are associated with proliferative diabetic retinopathy and macular oedema. *Diabetologia.* 2004 Aug; 47(8):1376–9. [PubMed: 15258735]
24. Zhang X, Frischmann M, Kientsch-Engel R, Steinmann K, Stopper H, Niwa T, et al. Two immunochemical assays to measure advanced glycation end-products in serum from dialysis patients. *Clin Chem Lab Med.* 2005; 43(5):503–11. [PubMed: 15899672]
25. Maggio M, Basaria S, Ble A, Lauretani F, Bandinelli S, Ceda GP, et al. Correlation between testosterone and the inflammatory marker soluble interleukin-6 receptor in older men. *The Journal of clinical endocrinology and metabolism.* 2006 Jan; 91(1):345–7. [PubMed: 16263825]
26. Blanc B, Finch CA, Hallberg L, Herbert V, Lawkowicz W, Layrisse M, et al. Nutritional anaemias. Report of a WHO scientific group. *World Health Organization technical report series.* 1968; 405:5–37. [PubMed: 4975372]
27. Ando K, Beppu M, Kikugawa K, Nagai R, Horiuchi S. Membrane proteins of human erythrocytes are modified by advanced glycation end products during aging in the circulation. *Biochem Biophys Res Commun.* 1999 Apr 29; 258(1):123–7. [PubMed: 10222246]
28. Iwata H, Ukeda H, Maruyama T, Fujino T, Sawamura M. Effect of carbonyl compounds on red blood cells deformability. *Biochem Biophys Res Commun.* 2004 Aug 27; 321(3):700–6. [PubMed: 15358163]
29. Wautier JL, Wautier MP, Schmidt AM, Anderson GM, Hori O, Zoukourian C, et al. Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel wall via a specific receptor inducing oxidant stress in the vasculature: a link between surface-associated AGEs and diabetic complications. *Proc Natl Acad Sci U S A.* 1994 Aug 2; 91(16):7742–6. [PubMed: 8052654]
30. Basta G. Receptor for advanced glycation endproducts and atherosclerosis: From basic mechanisms to clinical implications. *Atherosclerosis.* 2008 Jan; 196(1):9–21. [PubMed: 17826783]
31. Yan SF, Ramasamy R, Schmidt AM. Soluble RAGE: Therapy and biomarker in unraveling the RAGE axis in chronic disease and aging. *Biochemical pharmacology.* Jan 22.
32. Lu J, Holmgren A. Selenoproteins. *J Biol Chem.* 2009 Jan 9; 284(2):723–7. [PubMed: 18757362]

33. Hatanaka N, Nakaden H, Yamamoto Y, Matsuo S, Fujikawa T, Matsusue S. Selenium kinetics and changes in glutathione peroxidase activities in patients receiving long-term parenteral nutrition and effects of supplementation with selenite. *Nutrition*. 2000 Jan; 16(1):22–6. [PubMed: 10674230]
34. Espinoza SE, Guo H, Fedarko N, DeZern A, Fried LP, Xue QL, et al. Glutathione peroxidase enzyme activity in aging. *J Gerontol A Biol Sci Med Sci*. 2008 May; 63(5):505–9. [PubMed: 18511755]
35. Marinkovic D, Zhang X, Yalcin S, Luciano JP, Brugnara C, Huber T, et al. Foxo3 is required for the regulation of oxidative stress in erythropoiesis. *J Clin Invest*. 2007 Aug; 117(8):2133–44. [PubMed: 17671650]
36. Friedman JS, Rebel VI, Derby R, Bell K, Huang TT, Kuypers FA, et al. Absence of mitochondrial superoxide dismutase results in a murine hemolytic anemia responsive to therapy with a catalytic antioxidant. *J Exp Med*. 2001 Apr 16; 193(8):925–34. [PubMed: 11304553]
37. Mosoni L, Balage M, Vazeille E, Combaret L, Morand C, Zagol-Ikapitte I, et al. Antioxidant supplementation had positive effects in old rat muscle, but through better oxidative status in other organs. *Nutrition*. 2010 Nov-Dec; 26(11–12):1157–62. [PubMed: 20080031]
38. Semba RD, Bandinelli S, Sun K, Guralnik JM, Ferrucci L. Plasma carboxymethyl-lysine, an advanced glycation end product, and all-cause and cardiovascular disease mortality in older community-dwelling adults. *J Am Geriatr Soc*. 2009 Oct; 57(10):1874–80. [PubMed: 19682127]
39. Lauretani F, Semba RD, Bandinelli S, Ray AL, Ruggiero C, Cherubini A, et al. Low plasma selenium concentrations and mortality among older community-dwelling adults: the InCHIANTI Study. *Aging clinical and experimental research*. 2008 Apr; 20(2):153–8. [PubMed: 18431083]
40. Patel KV, Semba RD, Ferrucci L, Newman AB, Fried LP, Wallace RB, et al. Red cell distribution width and mortality in older adults: a meta-analysis. *J Gerontol A Biol Sci Med Sci*. Mar; 65(3): 258–65. [PubMed: 19880817]
41. Uribarri J, Peppia M, Cai W, Goldberg T, Lu M, Baliga S, et al. Dietary glycotoxins correlate with circulating advanced glycation end product levels in renal failure patients. *Am J Kidney Dis*. 2003 Sep; 42(3):532–8. [PubMed: 12955681]

Table 1

Relationship between demographic and other factors with prevalent anemia at enrollment in adults, aged 65 years and older in the InCHIANTI study

Characteristic ^I	Anemic (n = 120)	Non-anemic (n = 916)	P
Age (years)	83.0 (74.0, 88.5)	73.0 (69.0, 78.0)	<0.001
Female (%)	57.5	55.8	0.7
Education (years)	5.0 (3.0, 5.0)	5.0 (4.0, 6.0)	<0.001
Current smoking (%)	9.2	14.4	0.1
Body mass index (kg/m ²)	25.2 (22.8, 28.1)	27.3 (24.8, 30.1)	<0.001
Mini-Mental State Exam Score <24 (%)	51.7	26.4	<0.001
Ferritin <12 µg/L (%)	15.0	1.8	<0.001
Red cell distribution width (%)	14.4 (13.7, 16.0)	13.5 (13.1, 14.0)	<0.001
Folate <5.89 nmol/L (%)	37.7	30.8	0.1
Vitamin B ₁₂ <200 pg/mL (%)	16.7	10.5	0.05
Total testosterone, lowest quartile (%)	16.1	9.8	0.006
Bioavailable testosterone, lowest quartile (%)	40.9	23.0	<0.001
Carboxymethyl-lysine, highest quartile (%)	38.5	23.2	<0.001
Selenium, lowest quartile (%)	39.2	23.3	<0.001
Serum IL-6 > 2.5 pg/mL (%)	74.1	59.0	0.002
C-reactive protein > 5 mg/L (%)	38.3	28.6	0.03
Hypertension (%)	52.5	46.7	0.2
Angina (%)	7.5	4.0	0.08
Heart failure (%)	8.3	4.9	0.1
Stroke (%)	16.7	3.8	<0.001
Peripheral artery disease (%)	9.2	5.8	0.2
Diabetes mellitus (%)	11.7	13.0	0.7
Cancer (%)	8.3	6.1	0.4
Depression (%)	30.7	20.3	0.02
Chronic kidney disease (%)	28.3	13.8	<0.001

^IPercent or median (25th, 75th percentile).

Multivariate Cox proportional hazards models of risk factors for incident anemia over six years of follow-up among adults aged 65 years and older in the InCHIANTI study

Table 2

Characteristic	Model 1, adjusted for age, education, BML, and MMSE score			Model 2, adjusted for age, education, BML, MMSE score, IL-6, CRP, ferritin, RDW, vitamin B ₁₂ , and bioactive testosterone			Model 3, adjusted for age, education, BML, MMSE score, IL-6, CRP, ferritin, RDW, vitamin B ₁₂ , bioactive testosterone, stroke, depression, and chronic kidney disease		
	H.R.	95% C.I.	P	H.R.	95% C.I.	P	H.R.	95% C.I.	P
CML, highest quartile	1.54	1.02, 2.33	0.04	1.69	1.10, 2.59	0.02	1.67	1.07, 2.59	0.02
Selenium, lowest quartile	1.66	1.01, 2.50	0.04	1.50	0.98, 2.29	0.06	1.55	1.01, 2.38	0.05