Published in final edited form as:
Clin Nutr. 2010 October ; 29(5): 600–604. doi:10.1016/j.clnu.2010.03.001.

Serum antioxidants and inflammation predict red cell distribution width in older women: the Women's Health and Aging Study I

Richard D. Semba, M.D., M.P.H.[1], **Kushang V. Patel, Ph.D., M.P.H**[2], **Luigi Ferrucci, M.D., Ph.D.**[3], **Kai Sun, M.S.**[1], **Cindy N. Roy, Ph.D.**[1], **Jack M. Guralnik, M.D., Ph.D.**[2], and **Linda P. Fried, M.D., M.P.H.**[1]

[1]Johns Hopkins University School of Medicine, Baltimore, Maryland [2]Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, Bethesda, Maryland [3]Clinical Research Branch, National Institute on Aging, Baltimore, Maryland

Abstract

Background & Aims—Red cell distribution width (RDW), a measure of heterogeneity in the size of circulating erythrocytes, is associated with some chronic diseases and predicts mortality. Although oxidative damage and inflammation have been theorized to affect RDW, the relationships of antioxidants and inflammation with RDW have not been well characterized. The aims were to determine whether total serum carotenoids, α-tocopherol, selenium, protein carbonyls, and interleukin-6 (IL-6) are associated with RDW and predict RDW over time.

Methods—RDW was measured at baseline, 12 months, and 24 months follow-up in 786 moderately to severely disabled community-dwelling women, aged ≥65 years, in the Women's Health and Aging Study I in Baltimore, Maryland.

Results—Selenium was significantly associated with RDW at baseline and predicted RDW over two years' follow-up in separate multivariate mixed effects models that adjusted for other covariates. As expected, the addition of IL-6 to the models attenuated the association of serum selenium with RDW, as low antioxidant levels are known to upregulate IL-6. Total carotenoids were associated with RDW at baseline and one year follow-up. Protein carbonyls and α-tocopherol were not significantly associated with RDW.

Conclusion—Serum selenium is an independent predictor of RDW and may potentially mediate effects on RDW through IL-6.

Keywords

carotenoids; interleukin-6; oxidative stress; red cell distribution width; selenium; vitamin E

Correspondence to: Dr. Richard Semba, 550 N. Broadway, Suite 700, Baltimore, MD 21205. Tel. (410) 955-3572, Fax (410) 955-0629, rdsemba@jhmi.edu.

Conflict of Interest The authors have no conflict of interest.

Introduction

Red blood cell distribution width (RDW) is a measure of the variability in size of circulating erythrocytes and a parameter that is routinely reported as part of a complete blood count. Higher RDW values indicate greater heterogeneity in the size of circulating erythrocytes. Recently, elevated RDW was shown to predict mortality with great consistency across several different study populations.[1] The biological mechanisms that underlie the association of RDW with survival are unknown. RDW is elevated under conditions of erythropoietic stress, such as during iron, vitamin B_{12}, and folate deficiencies. Oxidative stress and inflammation have been suggested to influence RDW,[1] but the relationships of biomarkers of oxidative stress and inflammation with RDW have not been well characterized.

Oxidative stress refers to the condition in which the balance between oxidants and antioxidant defenses is upset and excess reactive oxygen species cause oxidative damage to nucleic acids, proteins, and lipids. Oxidative stress is related to red cell survival[2] and could be a possible mechanism for RDW. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are important antioxidant enzymes contained in erythrocytes. Mice with transplanted hemopoietic stem cells from SOD knockout mice had decreased red cell survival, and antioxidant treatment of the mice increased red cell survival.[2] In humans with selenium deficiency, selenium supplementation increased GSH-Px activity in erythrocytes.[3,4] The antioxidant defense system in humans includes carotenoids, α-tocopherol (vitamin E), and selenium. The six major dietary carotenoids found in the blood are α-carotene, β-carotene, α-cryptoxanthin, lutein, zeaxanthin, and lycopene. Alpha-tocopherol is a chain-breaking antioxidant that protects polyunsaturated fatty acids within membrane phosopholipids and plasma proteins by scavenging peroxyl radicals. Selenium is an essential trace element and normal constituent of the diet. The biochemical functions of selenium are related to its role in selenoproteins, and several of these selenoproteins are antioxidant enzymes, such as glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase, and thioredoxin reductase.

Reactive oxygen species have an extremely short half-life and are difficult to measure in humans, but it is possible to measure the damage that oxidative stress causes to protein, lipids, and DNA. Protein carbonyls represent several pathways of oxidative protein damage and are the most studied marker of protein oxidation in epidemiologic studies.[5] Inadequately opposed oxidative stress is suggested by low levels of circulating antioxidants. Oxidative stress and inflammation are often closely related because reactive oxygen species play a role in the activation of nuclear factor kappa B (NF-κB), a transcription factor that stimulates the expression of cytokines involved in the inflammatory process, such as interleukin-6 (IL-6).[6]

We hypothesized that RDW was associated with serum antioxidant concentrations and inflammation. In order to address this hypothesis, we examined the relationship of serum total carotenoids, α-tocopherol, selenium, and IL-6 with RDW in a cohort study of older, community-dwelling women.

Methods

Study participants

Subjects in this study were women, aged 65 and older, 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 the one third most disabled older 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.[7] Women were screened to identify self-reported

physical disability that was categorized into four domains. The domains of disability were ascertained in a 20-30 minute home interview that included questions related to (1) mobility and exercise tolerance, i.e., 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, i.e., 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, i.e., using the telephone, doing light housework, preparing your own meals, shopping for personal items), and (4) basic self-care tasks (a subset of non-mobility dependent activities of daily living, i.e., bathing or showering, dressing, eating, using the toilet). WHAS I enrolled women 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.[7]

Standardized questionnaires were administered in the participant's home by trained interviewers. Mini-Mental State Examination (MMSE) was recorded.[7] Medication use was recorded. 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 years or more than 12 years as the highest level of formal education achieved. Two weeks later, a trained registered full-time study nurse conducted an examination of each study participant in her home, using a standardized protocol that included physical performance measures and a standardized physical examination. Approximately 75% of women also consented to phlebotomy performed during a separate visit by a trained phlebotomist who followed a standardized protocol. Further details on the methods and sampling design of the WHAS studies are published elsewhere.[7] Women were allowed to participate after written, informed consent. The study protocol was approved by the Johns Hopkins School of Medicine Institutional Review Board.

Laboratory studies

There were 1002 women enrolled in the Women's Health and Aging Study I, of whom 786 women participated in the blood drawing and had red cell distribution width measurements at baseline. There were no significant differences in race or body mass index between those who did and did not participate in the blood drawing, but women who did and did not participate in the blood drawing were different by age (77.4 vs 80.7 years, respectively, $P < 0.0001$). Non-fasting blood samples were obtained by venipuncture between 9 AM and 2 PM. Processing, aliquoting, and freezing were carried out at the Core Genetics Laboratory of The Johns Hopkins University School of Medicine following a standardized protocol. Blood samples were delivered to Quest Diagnostics Laboratories (Teterboro, New Jersey) for complete blood count, ferritin, vitamin B_{12}, and folate measurements. Iron deficiency was defined as serum ferritin <12 μg/L.[8] Vitamin B_{12} deficiency was defined as a concentration <200 pg/mL and folate deficiency as a concentration <5.89 nmol/L.[9] Serum aliquots were stored continuously at −70° C until the time of analyses for serum carotenoids, tocopherols, selenium, protein carbonyls and interleukin-6.

Serum carotenoids and α-tocopherol were measured by high performance liquid chromatography (HPLC).[10] Total carotenoids were calculated as the sum of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene in μmol/L. Within-run and between-run coefficients of variation, respectively, were 10.7 and 23.9% for α-carotene, 7.0 and 19.1% for β-carotene, 4.7 and 8.5% for β-cryptoxanthin, 4.1 and 4.6% for lutein/zeaxanthin, 10.0 and 14.0% for lycopene, and 4.1 and 9.7% for α-tocopherol. Plasma selenium was measured by graphite furnace atomic absorption spectrometry using a Perkin Elmer AAnalyst 600 with Zeeman background correction. Samples were diluted 1:4 with a

triton-X (Sigma Chemical, St. Louis, MO) and nitric acid solution (Fisher Scientific, Pittsburgh, PA), and the matrix modifier was a palladium and magnesium nitrate solution (both Perkin Elmer, Norwalk, CT). The instrument was calibrated daily using known plasma selenium standards (UTAK Laboratories, Inc., Valencia, CA). Within-run and between-run coefficients of variation were 5.8% and 4.8%, respectively.

Serum protein carbonyls were measured using a commercial ELISA (Zentech PC Test, Protein Carbonyl Enzyme Immuno-Assay Kit, Zenith Technologies, Dunedin, NZ). Protein carbonyls are stable under long term storage at −70° C.[5] The assay has a minimum detectability of 0.02 nmol/mg protein, which is well below that range found in healthy human controls. Intra-assay and interassay CVs for protein carbonyl measurements were 10.1% and 18.2%, respectively. Serum IL-6 was measured using a commercial ELISA (Quantikine Human IL-6, R & D Systems, Minneapolis, MN). The minimum detection limit for the IL-6 ELISA reported by the manufacturer is 0.039 pg/mL. Intra-assay and interassay CVs for IL-6 measurements were 4% and 6%, respectively.

Statistical analysis

Mean and standard deviation were used to describe continuous variables in the study population. Log transformation of skewed variables was used to achieve a more normal distribution. Body mass index was categorized as underweight (<18.5 kg/m^2), normal range (18.5-24.9 kg/m^2), overweight (≥25-29.9 kg/m^2) and obese (≥30 kg/m^2). Renal insufficiency was defined as estimated glomerular filtration rate of <60 mL/min/1.73 m^2 using the four-variable Modification of Diet in Renal Disease Study equation of Levey and colleagues.[11] Univariate and multivariate linear regression analysis was used to examine the relationship between antioxidants and other factors where log RDW at baseline was the dependent variable. The relationship between antioxidants and inflammation at baseline and RDW over time were examined using mixed effects models.[12] Covariates were included in the multivariate models based upon clinical importance and the significance of the association in the univariate analyses. The level of significance in this study was P <0.05. All analyses were conducted using SAS version 9.13 (SAS Institute, Cary, NC).

Results

The demographic and disease characteristics of the 786 women by quartile of RDW are shown in Table 1. The relationships of serum antioxidants and other factors with RDW at baseline are shown in univariate linear regression models in Table 2. White race, total serum carotenoids, α-tocopherol, and selenium were significantly associated with a decrease in RDW. Iron deficiency, folate deficiency, IL-6, and heart failure were significantly associated with an increase in RDW. Age, education, BMI, smoking, MMSE score, vitamin B_{12} deficiency, serum protein carbonyls, hypertension, angina, peripheral artery disease, stroke, diabetes, chronic obstructive pulmonary disease, depression, cancer, and renal insufficiency were not significantly associated with RDW. In a cross-sectional analysis at baseline, total serum carotenoids and selenium were significantly associated with RDW in separate multivariate linear regression models that adjusted for race, and additionally for heart failure, iron and folate deficiencies (Table 3). The addition of IL-6 to the final model attenuated the association of total serum carotenoids and selenium with RDW.

Total serum carotenoids and selenium were significantly associated with RDW at baseline in separate multivariate random effects models that adjusted for race, heart failure, folate, and ferritin (Table 4). Selenium was an independent predictor of RDW at 12 and 24 months' follow-up visits, and the addition of IL-6 to the final model attenuated the association of serum selenium with RDW. Total serum carotenoids were significantly associated with RDW at baseline and the 12 month follow-up visit but not the 24 month follow-up visit.

Discussion

The present study shows that serum selenium is independently associated with RDW and predicts RDW over a two year period in older, disabled community-dwelling women. To our knowledge, this is the first study to show an association between selenium and RDW. The results suggest that oxidative stress may be a potential underlying biological mechanism for increased RDW. The relationship of serum selenium with RDW was attenuated when IL-6 was included in the multivariate models, and both serum carotenoids and selenium have been shown to predict changes in IL-6 over time.[13] Total serum carotenoids were independently associated with RDW at baseline and predicted RDW at the 12 month but not the 24 month follow-up.

Whether elevated RDW is a marker for low circulating total carotenoid and selenium concentrations is not clear. As noted previously, elevated RDW is a strong predictor of mortality.[1] Both low total serum carotenoids and low serum selenium concentrations are also strong predictors of mortality in older community-dwelling adults. Older community-dwelling adults with low total serum carotenoids are at higher risk of mortality, as shown in studies from the Netherlands,[14] the United States,[13] France,[15] and Italy.[16] Low serum selenium concentrations were independent predictors of mortality among older community-dwelling adults in France,[17] the United States,[13] and Italy.[18]

Serum selenium and carotenoids could theoretically protect erythrocytes from increased RDW by protecting erythrocytes from oxidative damage. Oxidative stress increases the fragility of red blood cells.[19] Oxidative stress also decreases the rate of erythroid maturation and decreases erythrocyte lifespan.[20] Beta-carotene supplementation has been shown to protect erythrocytes from the increased osmotic fragility that is found in zinc-deficient rats.[21] Glutathione peroxidase protects erythrocytes from oxidative damage, and in humans, selenium supplementation has been shown to increase glutathione peroxidase activities in erythrocytes.[3,4,22] Although α-tocopherol has been shown to protect erythrocytes from oxidative damage *in vitro*,[23] no significant relationship was found between serum α-tocopherol concentrations and RDW in the present study. Protein carbonyls were not associated with RDW and may represent a different pathway of oxidative stress that is not affected by selenium. Dietary selenium supplementation has been shown to have no effect upon protein carbonyls.[24]

Increased RDW has been reported in adults with impaired renal function,[25] inflammatory bowel disease,[26] and poor pulmonary function,[27] three conditions which are associated with low antioxidant nutrients. The primary source of plasma glutathione peroxidase is the kidney,[28] and plasma selenium, plasma glutathione peroxidase, and glutathione peroxidase activity in erythrocytes have been strongly correlated with stages of chronic renal failure.[29] Inflammatory bowel disease is associated with lower circulating carotenoids and increased oxidative stress.[30,31] Data from the Third National Health and Nutrition Examination Survey (NHANES III) shows that higher serum selenium and other antioxidant nutrients are associated with better pulmonary function.[32] Whether poor antioxidant nutrient status can explain the relationship between certain disease states and RDW needs to be explored in future studies.

The present study was limited to moderately to severe disabled women living in the community, and these findings cannot necessarily be generalized to less disabled women or to older men. Indeed, according to data from the NHANES III,[1] RDW levels were lower, on average, in the general population of adults aged 45 and older (median = 13.2%) than in the current study (median = 14.3%), possibly reflecting higher disease burden among older disabled women. In addition, other antioxidant nutrients which may protect erythrocytes

from oxidative damage, such as ascorbate and polyunsaturated fatty acids, were not measured in the present study and should be investigated in the future. The present study only involved the measurement of oxidative protein damage using protein carbonyls, and other types of oxidative damage to DNA and lipids was not addressed.

In conclusion, serum selenium was an independent predictor of RDW in older, moderately to severely disabled women living in the community. Antioxidant nutrient status may influence RDW and may potentially explain the association between elevated RDW and decreased survival. Future studies are needed that examine whether increased RDW is associated with decreased GSH-Px and SOD activity in erythrocytes or increased osmotic fragility, and whether increased RDW is associated with lower erythrocyte survival time.

Acknowledgments

This work was supported by National Institute on Aging Grants R01 AG027012, R01 AG029148 and the Intramural Research Program, National Institute on Aging, National Institutes of Health. The funding agency had no role in the conduct of the study or the data analysis. The study hypothesis and design were conceived by RDS, KVP, LF, and JMG. Funding was obtained by RDS, LF, and LPF. The data analysis was conducted by KS. The manuscript was written by RDS with substantial contributions to the paper by KVP, LF, KS, CNR, JMG, and LPF. All authors contributed to the final manuscript.

References

1. Patel KV, Semba RD, Ferrucci L, Newman AB, Fried LP, Wallace RB, Bandinelli S, Phillips CS, Yu B, Connelly S, Shlipak MG, Chaves PHM, Launer LJ, Ershler WB, Harris TB, Longo DL, Guralnik JM. Red cell distribution width and mortality in older adults: A meta-analysis. J Gerontol A Biol Sci Med Sci. 2009 Epub ahead of print.
2. Friedman JS, Lopez MF, Fleming MD, Rivera A, Martin FM, Welsh ML, Boyd A, Doctrow SR, Burakoff SJ. *SOD2*-deficiency anemia: protein oxidation and altered protein expression reveal targets of damage, stress response, and antioxidant responsiveness. Blood. 2004; 104:2565–2573. [PubMed: 15205258]
3. Alfthan G, Xu GL, Tan WH, Aro A, Wu J, Yang YX, Liang WS, Xue WL, Kong LH. Selenium supplementation of children in a selenium-deficient area in China. Blood selenium levels and glutathione peroxidase activities. Biol Trace Elem Res. 2000; 73:113–125. [PubMed: 11049204]
4. Thomson CD, Robinson MF, Butler JA, Whanger PD. Long-term supplementation with selenate and selenomethionine: selenium and glutathione peroxidase (*EC* 1.11.19) in blood components of New Zealand women. Br J Nutr. 1993; 69:577–588. [PubMed: 8490010]
5. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta. 2003; 329:23–38. [PubMed: 12589963]
6. Wang T, Zhang X, Li JJ. The role of NF-kappaB in the regulation of cell stress responses. Int Immunopharmacol. 2002; 2:1509–1520. [PubMed: 12433052]
7. Guralnik, JM.; Fried, LP.; Simosick, EM.; Kasper, JD.; Lafferty, ME., editors. The Women's Health and Aging study: Health and social characteristics of older women with disability. Bethesda, MD: National Institutes of Health, National Institute of Aging; 1995. NIH Pub No 95-4009
8. International Nutritional Anemia Consultative Group (INACG). A Report of the International Nutritional Anemia Consultative Group. Washington, D.C.: The Nutrition Foundation; 1985. Measurement of Iron Status.
9. 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; 104:2263–2268. [PubMed: 15238427]
10. Sowell AL, Huff DL, Yeager PR, Caudill SP, Gunter EW. Retinol, α-tocopherol, lutein/zeaxanthin, ß-cryptoxanthin, lycopene, α-carotene, *trans*-ß-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multiwavelength detection. Clin Chem. 1994; 40:411–416. [PubMed: 8131277]

11. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med. 1999; 130:461–470. [PubMed: 10075613]
12. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics. 1982; 38:963–974. [PubMed: 7168798]
13. Walston J, Xue Q, Semba RD, Ferrucci L, Cappola A, Ricks M, Guralnik J, Fried LP. Serum antioxidants, inflammation, and total mortality in older women. Am J Epidemiol. 2006; 163:18–26. [PubMed: 16306311]
14. Waart FG, Schouten EG, Stalenhoef AFH, Kok FJ. Serum carotenoids, α-tocopherol and mortality risk in a prospective study among Dutch elderly. Int J Epidemiology. 30; 2001:136–143.
15. Akbaraly TN, Favier A, Berr C. Total plasma carotenoids and mortality in the elderly: results of the Epidemiology of Vascular Ageing (EVA) study. Br J Nutr. 2008; 29:1.
16. Lauretani F, Semba RD, Dayhoff-Brannigan M, Corsi AM, Di Iorio A, Bandinelli S, Guralnik JM, Ferrucci L. Low total plasma carotenoids are independent predictors of mortality among older persons: the InCHIANTI study. Eur J Nutr. 2008; 47:517–525.
17. Akbaraly NT, Arnaud J, Hininger-Favier I, Gourlet V, Roussel AM, Berr C. Selenium and mortality in the elderly: results from the EVA study. Clin Chem. 2005; 51:2117–2123. [PubMed: 16123147]
18. Lauretani F, Semba RD, Bandinelli S, Ray AL, Ruggiero C, Cherubini A, Guralnik JM, Ferrucci L. Low plasma selenium concentrations and mortality among older community-dwelling adults: the InCHIANTI study. Aging Clin Exp Res. 2008; 20:153–158. [PubMed: 18431083]
19. Tozzi-Ciancarelli MG, Di Giulio A, Troiani-Sevi E, D'Alfonso A, Amicosante G, Oratore A. Human erythrocyte damage at the initial stages of oxidative stress. Cell Biophys. 1989; 15:225–234. [PubMed: 2480182]
20. Marinkovic D, Zhang X, Yalcin S, Luciano JP, Brugnara C, Huber T, Ghaffari S. Foxo3 is required for the regulation of oxidative stress in erythropoiesis. J Clin Invest. 2007; 117:2133–2144. [PubMed: 17671650]
21. Kraus A, Roth HP, Kirchgessner M. Supplementation with vitamin C, vitamin E or β-carotene influences osmotic fragility and oxidative damage of erythrocytes of zinc-deficient rats. J Nutr. 1997; 127:1290–1296. [PubMed: 9202082]
22. Thomson CD, Ong LK, Robinson MF. Effects of supplementation with high-selenium wheat bread on selenium, glutathione peroxidase and related enzymes in blood components of New Zealand residents. Am J Clin Nutr. 1985; 41:1015–1022. [PubMed: 3993605]
23. Durak D, Uzun FG, Kalender S, Ogutcu A, Uzunhisarcikli M, Kalender Y. Malathion-induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E in vitro. Environ Toxicol. 2009; 47:1903–1908.
24. Wu J, Salisbury C, Graham R, Lyons G, Fenech M. Increased consumption of wheat biofortified with selenium does not modify biomarkers of cancer risk, oxidative stress, or immune function in healthy Australian males. Environ Mol Mutagen. 2009; 50:489–501. [PubMed: 19365873]
25. Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, Gidi GC. Relationship between red blood cell distribution width and kidney function tests in a large cohort of unselected patients. Scand J Clin Lab Investig. 2008 Epub ahead of print.
26. Cakal B, Akoz AG, Ustundag Y, Yalinkilic M, Ulker A, Ankarali H. Red cell distribution width for assessment of activity of inflammatory bowel disease. Dig Dis Sci. 2008 Epub ahead of print.
27. Grant BJB, Kudalkar DP, Muti P, McCann SE, Trevisan M, Freudenheim JL, Schünemann HJ. Relation between lung function and RBC distribution width in a population-based study. Chest. 2003; 124:494–500. [PubMed: 12907534]
28. Avissar N, Ornt DB, Yagil Y, Horowitz S, Watkins RH, Kerl EA, Takahashi K, Palmer IS, Cohen HJ. Human kidney proximal tubules are the main source of plasma glutathione peroxidase. Am J Physiol. 1994; 35:C367–C375. [PubMed: 8141250]
29. Zachara BA, Salak A, Koterska D, Manitius J, Wasowicz W. Selenium and glutathione peroxidases in blood of patients with different stages of chronic renal failure. J Trace Elem Med Biol. 2004; 17:291–299. [PubMed: 15139391]

30. D'Odorico A, Bortolan S, Cardin R, D'Inca' R, Martines D, Ferronato A, Sturniolo GC. Reduced plasma antioxidant concentrations and increased oxidative DNA damage in inflammatory bowel disease. Scand J Gastroenterol. 2001; 36:1289–1294. [PubMed: 11761019]

31. Hengstermann S, Valentini L, Schaper L, Buning C, Koernicke T, Maritschnegg M, Buhner S, Tillinger W, Regano N, Guglielmi F, Winklhofer-Roob BM, Lochs H. Altered status of antioxidant vitamins and fatty acids in patients with inactive inflammatory bowel disease. Clin Nutr. 2008; 27:571–578. [PubMed: 18316141]

32. McKeever TM, Lewis SA, Smit HA, Burney P, Cassano PA, Britton J. A multivariate analysis of serum nutrient levels and lung function. Resp Res. 2008; 9:67.

Table 1

Characteristics of women in the Women's Health and Aging Study I by quartile[1] of RDW

Characteristic[2]		Quartile 1 (Lowest)	Quartile 2	Quartile 3	Quartile 4 (Highest)	*P*
Age, years		77.0 (8.1)	78.2 (7.5)	77.8 (7.7)	77.7 (7.8)	0.40
Race	White	79.8	80.3	73.7	55.3	<0.0001
	Other	20.2	19.7	26.3	44.7	
Education <12 years (%)		60.4	65.5	67.9	64.8	0.45
Body mass index (kg/m^2) (%)	<18.5	4.3	4.4	2.2	4.7	0.04
	18.5-24.9	27.1	24.2	31.3	18.6	
	25.0-29.9	40.9	33.8	33.5	33.1	
	≥30	27.7	37.5	33.0	43.6	
Current smoker (%)		9.4	8.8	12.2	14.9	0.24
Mini-Mental Status Exam score <24 (%)		14.3	12.2	21.1	18.6	0.10
Log total carotenoids (μmol/L)		0.43 (0.48)	0.35 (0.49)	0.34 (0.48)	0.28 (0.52)	0.07
Log alpha-tocopherol (μmol/L)		0.82 (0.89)	0.82 (0.88)	0.75 0.97)	0.61 (1.04)	0.20
Selenium (μg/dL)		120 (17)	119 (18)	117 (19)	114 (19)	0.0008
Log ferritin (μg/L)		4.5 (0.9)	4.5 (0.9)	4.3 (0.8)	4.2 (1.1)	0.06
Ferritin <12 μg/L (%)		0.5	0.7	1.9	7.0	0.0002
Log vitamin B_{12} (pg/mL)		6.1 (0.5)	6.0 (0.5)	6.0 (0.5)	6.1 (0.5)	0.26
Vitamin B_{12} <200 pg/mL (%)		6.6	7.6	6.8	5.4	0.87
Log folate (nmol/L)		2.4 (0.6)	2.3 (0.6)	2.2 (0.6)	2.1 (0.7)	0.002
Folate <5.89 nmol/L (%)		0	0	0.9	2.7	0.03
Log protein carbonyls (nmol/mg)		-2.48 (0.49)	-2.46 (0.44)	-2.43 (0.46)	-2.45 (0.55)	0.73
Log IL-6 (pg/mL)		0.73 (0.68)	0.84 (0.60)	0.98 (0.64)	1.12 (0.66)	<0.0001
Hypertension		56.6	59.8	53.5	63.8	0.19
Angina		25.6	23.1	23.9	20.7	0.72
Heart failure		8.8	4.7	14.1	14.4	0.01
Peripheral artery disease		21.2	21.1	22.1	23.9	0.91
Stroke		3.4	5.4	6.6	6.4	0.49

Characteristic[2]	Quartile 1 (Lowest)	Quartile 2	Quartile 3	Quartile 4 (Highest)	*P*
Diabetes mellitus	17.2	16.3	18.3	14.9	0.83
Chronic obstructive pulmonary disease	28.1	30.6	26.3	28.2	0.84
Depression	19.7	16.3	15.5	18.1	0.69
Cancer	10.8	13.6	11.3	11.7	0.87
Renal insufficiency	56.7	53.4	52.2	57.6	0.42

[1] Quartile cut-offs for RDW were 13.4, 14.0, and 14.9%.

[2] Mean (SD) for continuous variables or percent or participants with specific characteristic as noted.

Table 2

Univariate relationships between serum antioxidants and other factors with log RDW at baseline among participants in the Women's Health and Aging Study I

Characteristic	Standardized Beta	SE	*P*
Age (years)	0.036	0.036	0.32
Race, white	-0.394	0.080	<0.0001
Education <12 years	0.106	0.076	0.16
Body mass index (kg/m^2)	0.044	0.038	0.25
Current smoker	0.156	0.114	0.17
Mini-Mental State Exam score <24	0.110	0.097	0.26
Log total serum carotenoids (μmol/L)	-0.142	0.036	0.0001
Log serum α-tocopherol (μmol/L)	-0.137	0.036	0.0002
Serum selenium (μg/dL)	-0.143	0.037	0.0001
Ferritin <12 μg/L	0.117	0.023	<0.0001
Vitamin B_{12} <200 pg/mL	-0.007	0.015	0.66
Folate <5.89 nmol/L	0.107	0.039	0.007
Log serum IL-6 (pg/mL)	0.225	0.035	<0.0001
Log serum protein carbonyls (nmol/mg)	0.053	0.037	0.15
Hypertension	0.102	0.074	0.17
Angina	-0.091	0.086	0.29
Heart failure	0.343	0.116	0.003
Peripheral artery disease	0.059	0.087	0.49
Stroke	0.064	0.161	0.68
Diabetes	-0.085	0.097	0.38
Chronic obstructive pulmonary disease	0.042	0.081	0.60
Depression	-0.006	0.096	0.95
Cancer	0.076	0.113	0.51
Renal insufficiency	-0.029	0.075	0.70

Table 3

Multivariate linear regression models of the relationship of serum antioxidants log RDW width at baseline in participants in the Women's Health and Aging Study I[1]

	Model adjusted for race			Model adjusted for race, heart failure			Model adjusted for age, race, heart failure, folate, ferritin			Model adjusted for age, race, heart failure, folate, ferritin, IL-6		
	Beta[2]	SE	*P*	Beta[2]	SE	*P*	Beta[2]	SE	*P*	Beta[2]	SE	*P*
Log total carotenoids (µmol/L)	-0.141	0.045	0.002	-0.135	0.046	0.003	-0.138	0.044	0.002	-0.087	0.045	0.05
Log alpha-tocopherol (µmol/L)	-0.029	0.043	0.49	-0.025	0.043	0.55	-0.021	0.042	0.62	-0.028	0.042	0.50
Selenium (µg/dL)	-0.104	0.038	0.006	-0.100	0.038	0.008	-0.092	0.037	0.01	-0.061	0.037	0.10

[1] Separate multivariate linear regression models shown for antioxidants and IL-6 in which log RDW is the dependent variable.

[2] Standardized betas.

Table 4

Multivariate linear mixed-effects models of the relationship of serum antioxidants and IL-6 at baseline with log RDW over 24 months of follow-up among participants in the Women's Health and Aging Study I[1]

Analyte	Time points	Model adjusted for race, heart failure, folate, ferritin			Model adjusted for race, education, heart failure, folate, ferritin, IL-6		
		Beta[2]	SE	*P*	Beta[2]	SE	*P*
Log total carotenoids (μmol/L)	Baseline	-0.140	0.043	0.001	-0.090	0.045	0.04
	12-month follow-up	-0.095	0.049	0.05	-0.058	0.050	0.24
	24-month follow-up	-0.064	0.058	0.27	-0.037	0.060	0.53
Log alpha-tocopherol (μmol/L)	Baseline	-0.018	0.042	0.65	-0.027	0.041	0.51
	12-month follow-up	-0.021	0.048	0.65	-0.026	0.047	0.57
	24-month follow-up	-0.082	0.065	0.19	0.077	0.065	0.23
Selenium (μg/dL)	Baseline	-0.093	0.036	0.01	-0.063	0.036	0.08
	12-month follow-up	-0.146	0.041	0.0004	-0.112	0.041	0.006
	24-month follow-up	-0.116	0.049	0.02	-0.092	0.050	0.06

[1] Multivariate linear mixed-effects models where RDW over 24 months of follow-up is the dependent variable.

[2] Standardized betas.