Published in final edited form as:

Am J Ophthalmol. 2007 February ; 143(2): 206–211.

Effects of Long-term Zinc Supplementation on Plasma Thiol Metabolites and Redox Status in Patients with Age-related Macular Degeneration

Paul Sternberg Jr., MD, Khoi-Nguyen Ha, BS, Siobhan E. Moriarty-Craige, BS, Michael Lynn, MS, Susan Bressler, MD, Gary Gensler, MS, and Dean P. Jones, PhD

From the Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, TN (P.S., K.N.H.); Departments of Medicine, Emory University School of Medicine, Atlanta, GA (S.M.-C., D.P.J.); Department of Biostatistics, Rollins School of Public Health of Emory University, Atlanta, GA (M.L.); Wilmer Ophthalmological Institute, Johns Hopkins University School of Medicine, Baltimore, MD (S.B.); the EMMES Corporation, Rockville, MD (G.G.).

Abstract

PURPOSE—To determine the effects of zinc supplementation on plasma thiol metabolites and their redox status in a cohort of patients with age-related macular degeneration (AMD).

DESIGN—Randomized clinical trial that evaluated the effects of high doses of zinc and antioxidants on plasma biomarkers of oxidative stress.

METHODS—This was an ancillary study of the Age-Related Eye Disease Study (AREDS). Subjects with AMD were randomized to one of four treatment groups: 1) antioxidants (vitamin C, 500 mg; vitamin E, 400 IU; and beta carotene, 15 mg); 2) zinc (80 mg zinc oxide, 2 mg cupric oxide); 3) antioxidants plus zinc; or 4) placebo. At 20 and 80 months after randomization, blood specimens were collected and analyzed for glutathione (GSH), oxidized glutathione (GSSG), cysteine (Cys), and cystine (CySS).

RESULTS—Although zinc supplementation had no apparent effect on plasma thiol/disulfide redox status at the first blood draw, the group of patients receiving zinc supplementation at the second blood draw had significantly less CySS compared to those not receiving zinc (54.9 vs. 64.1 μ M; $p = 0.01$). There was a time-dependent oxidation of the plasma GHS pool and was not affected by zinc supplementation.

CONCLUSIONS—Because increased CySS level is associated with aging, oxidative stress, and age-related diseases, the apparent prevention of increased CySS by zinc supplementation warrants additional investigation.

INTRODUCTION

Accumulating evidence implicates that oxidative stress is involved in the pathogenesis of AMD; however, no definitive link has yet been established.^{1–3} A number of studies have evaluated the potential benefits of antioxidant supplementation in delaying the development and progression of AMD. Results from most observational studies have suggested that intake

Inquiries to: Paul Sternberg, Jr., M.D., Vanderbilt Eye Institute, 8000 Medical Center East, North Tower, Nashville, TN 37232-8808. Phone: (615) 936-1453; Fax: (615) 936-3497; email: paul.sternberg@vanderbilt.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.

of antioxidants is associated with a reduction in the risk of AMD development.^{4–6} By conducting a randomized, placebo-controlled, two-year clinical trial, Newsome et al showed that zinc supplementation reduced the risk of vision loss in patients with AMD.⁷ This early finding was supported by the AREDS,⁸ which showed that long-term (6.3 years) intake of zinc, alone or with antioxidants, significantly delayed the risk of progression from intermediate to advanced AMD and reduced the rate of moderate vision loss.

In addition to inspecting the visual function and retina, measurement of peripheral biomarkers is of great importance in monitoring the effects of long term dietary antioxidant supplementation. We and others have previously showed that the plasma thiol metabolites, glutathione (GSH) and cysteine (Cys), become more oxidized with age,^{9,10} oxidative stress,^{11,12} and age-related diseases.^{10,13,14} The levels of these metabolites and the redox status of the thiol/disulfide couples in the plasma are likely to be reliable markers of systemic oxidative stress and antioxidant defense.^{15–17} The tightly regulated extracellular redox environment is also important in maintaining a number of tissue and cell functions.^{18–21}

In this study, we analyzed plasma GSH/GSSG and Cys/CySS and their redox status in a subset of AREDS patients at both the Emory and Wilmer Eye Centers. Results showed that long-term zinc supplementation resulted in a decreased plasma CySS concentration, although the redox potential (E_h) of the Cys(s) pool was not affected. The increased tissue transport and utilization of CySS, potentially induced by zinc, may contribute to the beneficial effects in protecting against the pathogenesis of AMD.

METHODS

We obtained plasma samples from subjects who had been enrolled in the AREDS multi center clinical trial.⁸ This trial was designed to evaluate the effects of antioxidant and/or zinc supplementation on the progression of AMD in a cohort of individuals between age 55 to 80. All participants at the Emory and Wilmer Eye Centers who could be scheduled for blood collection during regular visit were included. Participants with AMD were randomly assigned to one of four treatment groups: 1) antioxidants (vitamin C, 500 mg; vitamin E, 400 IU; beta carotene, 15 mg); 2) zinc (zinc oxide, 80 mg; cupric oxide, 2 mg); 3) antioxidants plus zinc; or 4) placebo.⁸ At both study sites, blood specimens were obtained at two time points, an average of 1.7 and 6.7 years after enrollment. The study and recruitment procedures were approved by the Investigational Review Board of Emory University and approved as an ancillary study by the AREDS Executive Committee. Informed consent was obtained from all participants.

Blood (0.5 ml) was collected with syringes and 23-gauge heparinized butterfly needles and transferred immediately to microcentrifuge tubes containing 0.5 ml of bathophenanthroline disulfonate, a redox preservation solution.¹⁷ Tubes were then centrifuged to remove blood cells and 200 μ l of supernatant was transferred into 200 μ l of perchloric acid solution containing 0.2 M boric acid and 10 μ M γ -glutamylglutamate (internal standard). This procedure enables quantitative recovery of plasma glutathione (GSH), glutathione disulfide (GSSG), cysteine (Cys), and cystine (CySS) and minimizes variation in redox states between sampling and acidification.¹⁷ Samples were stored (for less than 2 months) at -80°C prior to derivatization and assayed with high performance liquid chromatography (HPLC) using fluorescence detection, as previously described.¹⁷

Sodium heparin, bathophenanthroline disulfonate sodium salt (BPDS), iodoacetic acid, dansyl chloride, L-serine, γ -glutamylglutamate, GSH, GSSG, Cys, CySS, and sodium acetate trihydrate were purchased from Sigma Chemical Corp. (St. Louis, MO). The mixed disulfide of Cys and GSH (CySSG) was purchased from Toronto Research Chemicals (Toronto, ON,

Canada). Boric acid (BA), sodium tetraborate, potassium tetraborate, perchloric acid (PCA), and acetic acid of reagent grade were purchased locally. Methanol, acetone, and chloroform were HPLC grade.

The redox potentials of the GSH/GSSG and Cys/CySS couples (E_h GSH and E_h Cys, respectively) were calculated using the Nernst equation:

$$E_h = E_o + \frac{RT}{nF} \ln \frac{[\text{disulfide}]}{[\text{thiol}]^2}$$

where R is the gas constant, T is the absolute temperature, n is the number of electrons transferred (for GSH and Cys it is 2), and F is the Faraday's constant. The standard potentials, E_o , at pH 7.4 for the Cys/CySS and GSH/GSSG couples are -250 mV and -264 mV, respectively.

For the purpose of this study, the four treatment groups were combined into two groups: a) zinc supplementation (combining Groups 2 and 3), and b) no zinc (combining Groups 1 and 4). Statistical comparisons of the means of the metabolites for the two treatments (zinc versus no zinc) at the two blood draws were made using a repeated measures analysis of variance model. Separate models were employed for each metabolite. The models were fit following a general linear mixed models approach using the Proc MIXED procedure of the Statistical Analysis System (SAS). Comparisons of the means of the two treatments at each blood draw and of the means of the last two blood draws for each treatment were made by estimates of the differences in the least squares means as provided by Proc MIXED. No adjustments were made for multiple comparisons. P-values less than 0.05 were considered statistically significant. Metabolites other than redox metabolites were transformed using the natural log function prior to fitting the model because of non-normal distributions. Summary statistics and graphs are based on the original untransformed variables.

RESULTS

SUBJECT DEMOGRAPHIC CHARACTERISTICS

To control for other factors (age, smoking, obesity, and diabetes) that may have an effect on the plasma thiols, we examined the average age of the patients, smoking habits, body mass index (BMI), and the presence of diabetes. In this study, a total of 312 AMD subjects were identified for the first blood draw, with 112 from Emory and 200 from Johns Hopkins. There were 82 AMD subjects for the second blood draw, with 51 from Emory and 31 from Johns Hopkins. There were no significant differences in the demographic characteristics between subjects receiving zinc supplementation and subjects not receiving zinc at either the first or second blood draw (Table 1). It should be noted that the second set of blood samples were initiated later at Johns Hopkins than at Emory and that the study ended before the scheduled ending date, leading to a higher percentage of subjects from Emory. Otherwise, the second blood draw group had comparable characteristics to that of the first blood draw group (Table 1). By design, the second blood draw was performed 80 months after randomization versus 20 months after randomization for the first draw. As such, there was a significant difference in patient age between draw 1 and draw 2 (71.4y versus 75.9y).

BIOCHEMICAL CHARACTERISTICS AT EACH BLOOD DRAW

To establish a baseline for comparison, we measured and compared plasma thiol metabolites and their redox status between the zinc supplemented and the no zinc groups. At the first blood draw, most subjects had already entered the study, had been randomized and were receiving zinc or placebo. There were no differences in any of the thiols, disulfides, or redox states

between the zinc supplemented and the no zinc groups (Table 2). At the second collection, no effects on either plasma GSH/GSSG or Cys/CySS redox status were seen. However, there was a decrease in the plasma CySS concentration in subjects who had received zinc supplementation as compared to those who had not received zinc (54.9 μ M vs. 64.1 μ M; $p = 0.01$, Fig. 1). In the plasma, CySS is 5 to 10 fold higher in concentration than the reduced form, Cys, therefore, we observed a concomitant decrease in the total Cys pool in the zinc treated group compared to the no zinc group (125.9 μ M vs. 142.1 μ M, $p = 0.01$). There were no significant differences in Cys concentration between the groups.

CHANGES OVER TIME OF THE BIOCHEMICAL CHARACTERISTICS

Over the five-year period, there was a significant oxidation of the plasma E_h GSH in both the zinc supplemented and no zinc groups ($p = .01$ and $p = .007$, respectively; Table 2). There was a slight but statistically significant decrease in plasma GSH concentration in the zinc supplemented group ($p = .05$) and a statistically significant increase in the plasma GSSG concentration in the no zinc group ($p = .001$). With zinc treatment, plasma CySS concentration decreased significantly from 58.3 μ M to 54.9 μ M ($p = 0.05$, Fig. 1). In contrast, plasma Cys concentration increased from 57.7 μ M to 67.1 μ M without zinc treatment. This was accompanied by a trend of increase in Total Cys from 125.9 μ M to 142.1 μ M ($p = .08$). There were no changes in the plasma E_h Cys in either group.

DISCUSSION

AMD is the leading cause of blindness in the elderly, affecting seven million Americans, 1.75 million of whom have more advanced disease associated with severe vision loss. It is estimated that the number of people with advanced AMD will double by the year 2020.²² Following AREDS, zinc and antioxidant supplementation became the only accepted preventive measure for atrophic or “dry” AMD. Despite the widespread intake of zinc, little is known about the biochemical effects elicited by zinc.

This study analyzes the effect of long-term zinc supplementation on plasma thiol metabolites and their redox status. There was a significant decrease in plasma CySS concentration in the zinc supplemented group. This decrease in CySS contributed to a decrease in the total plasma Cys concentration, which is comprised mainly of CySS. There were no significant differences in the plasma Cys/CySS redox state in both groups.

Previous studies suggest that, in hepatoma²³ and in ARPE-19 cells²⁴, zinc could activate the transcription factor Nrf2 and increase the expression of phase II detoxification genes controlled by the antioxidant response element (ARE). One of such genes is the cystine/glutamate exchanger, the X_c^- system, in which glutamate is exported from the cell in exchange for the uptake of cysteine.²⁵ The functional roles of X_c^- in controlling plasma and tissue CySS have been confirmed in knockout mice.²⁶ By activating the ARE/Nrf2 pathway, zinc supplementation may upregulate the cystine/glutamate exchanger (Fig. 2). Such effect will lead to increased tissue and cellular uptake of CySS and, consequently, decreased plasma CySS concentration.

In addition to be a potential biochemical marker of dietary supplementation, changes in the plasma CySS levels may directly regulate cellular functions. Previous studies demonstrate that variations of the extracellular Cys/CySS within physiologic range (-20 to -130 mV)¹⁰ can increase cell proliferation through an activation of the epidermal growth factor receptor (EGFR) and the p44/p42 MAPK pathway.¹⁸ In contrast, oxidation of the Cys/CySS stimulates monocyte adhesion to vascular endothelial cells through an upregulation of various adhesion molecules by an NF- κ B dependent pathway.²⁰ Jiang et al. showed that in more oxidized Cys/CySS environments, cultured human RPE cells are more susceptible to oxidant-induced

apoptosis.²¹ Data from the present study showed an about 10 μM differences (54.9 μM vs. 64.1 μM , zinc vs no zinc), which is about 15% of the normal plasma CySS concentration. How this magnitude of change may affect the RPE function remains to be determined by future studies.

This study has several limitations. Due to the small sample size, we were unable to adjust the analyses for either the AMD status or the supplementation compliance. Also, in order to achieve adequate statistical power, patients treated with zinc alone and zinc plus antioxidants were combined into the zinc treated group, while patients receiving antioxidants alone and placebo were grouped into the no zinc group. A prior study had shown that antioxidant vitamins inhibited the oxidation of thiol metabolites.²⁷ To definitely prove the interaction between zinc supplementation and plasma thiol/disulfide redox status, future studies with larger sample size will be needed.

Zinc is concentrated in the choriocapillaris, RPE, and retina.²⁸ The effect of zinc at the local level may be more pronounced than what can be measured systemically. In this report we have demonstrated that increased oral intake of zinc can modulate the plasma CySS concentration. If such change can be linked to visual function, we will have established a reliable peripheral marker of monitoring the therapeutic effects in AMD patients.

Acknowledgements

This project was supported by: NIH grants EY07892, EY08126, EY06360, Foundation Fighting Blindness, and Research to Prevent Blindness, Inc.

Financial Disclosure: None

References

1. Cai J, Nelson KC, Wu M, Sternberg P Jr, Jones DP. Oxidative damage and protection of the RPE. *Prog Retin Eye Res* 2000;19:205–221. [PubMed: 10674708]
2. Liang FQ, Godley BF. Oxidative stress-induced mitochondrial DNA damage in human retinal pigment epithelial cells: a possible mechanism for RPE aging and age-related macular degeneration. *Exp Eye Res* 2003;76:397–403. [PubMed: 12634104]
3. Beatty S, Koh H, Phil M, Henson D, Bouton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 2000;45:115–134. [PubMed: 11033038]
4. VandenLangenberg GM, Mares-Perlman JA, Klein R, et al. Association between antioxidant and zinc intake and the 5-year incidence of early age-related maculopathy in the Beaver Dam Eye Study. *Am J Epidemiol* 1998;148:204–214. [PubMed: 9676703]
5. Evans JR. Risk factors for age-related macular degeneration. *Prog Retin Eye Res* 2001;20:227–253. [PubMed: 11173253]
6. van Leeuwen R, Boekhoorn S, Vingerling JR, Witteman JC, Klaver CC, Hofman A, de Jong PT. Dietary intake of antioxidants and risk of age-related macular degeneration. *JAMA* 2005;28:3101–3107. [PubMed: 16380590]
7. Newsome DA, Swartz M, Leone MC, Elston RC, Miller E. Oral zinc in macular degeneration. *Arch Ophthalmol* 1988;106:192–198. [PubMed: 3277606]
8. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS Report No. 8. *Arch Ophthalmol* 2001;119:1417–1436. [PubMed: 11594942]
9. Jones DP, Carlson JL, Mody VC Jr, Cai J, Lynn MJ, Sternberg P Jr. Redox State of Glutathione in Human Plasma. Redox State of Glutathione in Human Plasma. *Free Radic Biol Med* 2000;28:625–635. [PubMed: 10719244]

10. Jones DP, Mody VC, Carlson JL, Lynn MJ, Sternberg P Jr. Redox Analysis of Human Plasma Allows Separation of Pro-oxidant Events of Aging from Decline in Antioxidant Defenses. *Free Radic Biol Med* 2002;33:1290–1300. [PubMed: 12398937]
11. Moriarty SE, Shah JH, Lynn M, Jiang S, Openo K, Jones DP, Sternberg P Jr. Oxidation of Glutathione and Cysteine in Human Plasma Associated with Smoking. *Free Radic Biol Med* 2003;35:1582–1588. [PubMed: 14680681]
12. Jonas CR, Puckett AB, Jones DP, Griffith DP, Szeszycki EE, Bergman GF, Furr CE, Tyre C, Carlson JL, Galloway JR, Blumberg JB, Ziegler TR. Plasma antioxidant status after high-dose chemotherapy: a randomized trial of parenteral nutrition in bone marrow transplantation patients. *Am J Clin Nutr* 2000;72:181–189. [PubMed: 10871578]
13. Samiec PS, Drews-Botsch C, Flagg EW, Kurtz JC, Sternberg P Jr, Reed RL, Jones DP. Glutathione in Human Plasma: Decline in Association with Aging, Age-Related Macular Degeneration, and Diabetes. *Free Radic Biol Med* 1998;24:699–704. [PubMed: 9586798]
14. Ashfaq S, Abramson JL, Jones DP, Rhodes SD, Weintraub WS, Hooper WC, Vaccarino V, Harrison DG, Quyyumi AA. The relationship between plasma levels of oxidized and reduced thiols and early atherosclerosis in healthy adults. *J Am Coll Cardiol* 2006;47:1005–1011. [PubMed: 16516085]
15. Jones DP. Extracellular Redox State: Refining the Definition of Oxidative Stress in Aging. *Rejuvenation Res* 2006;9:169–181. [PubMed: 16706639]
16. Pastore A, Federici G, Bertini E, Piemonte F. Analysis of glutathione: implication in redox and detoxification. *Clinica Chimica Acta* 2003;333:19–39.
17. Jones DP, Carlson JL, Sameic PS, Sternberg P Jr, Mody VC Jr, Reed RL, Brown LA. Glutathione measurement in human plasma: evaluation of sample collection, storage and derivatization conditions for analysis of dansyl derivatives by HPLC. *Clin Chim Acta* 1998;275:175–184. [PubMed: 9721075]
18. Nkabyo YS, Go Y-M, Ziegler TR, Jones DP. Extracellular cysteine/cystine redox regulates the p44/p42 MAPK pathway by metalloproteinase-dependent epidermal growth factor receptor signaling. *Am J Physiol Gastrointest Liver Physiol* 2005;289:G70–G80. [PubMed: 15746213]
19. Van Wart HE, Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci USA* 1990;87:5578–5582. [PubMed: 2164689]
20. Go Y-M, Jones DP. Intracellular Proatherogenic Events and Cell Adhesion Modulated by Extracellular Thiol/Disulfide Redox State. *Circulation* 2005;111:2973–2980. [PubMed: 15927968]
21. Jiang S, Moriarty-Craige SE, Orr M, Cai J, Sternberg P Jr, Jones DP. Oxidant-induced apoptosis in human retinal pigment epithelial cells: dependence on extracellular redox state. *Invest Ophthalmol Vis Sci* 2005;46:1054–1061. [PubMed: 15728565]
22. Prevalence of AMD Research Group. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol* 2004;122:564–572. [PubMed: 15078675]
23. Prestera T, Holtzclaw WD, Zhang Y, Talalay P. Chemical and molecular regulation of enzymes that detoxify carcinogens. *Proc Natl Acad Sci USA* 1993;90:2965–2969. [PubMed: 8385353]
24. Ha K-N, Chen Y, Cai J, Sternberg P Jr. Increased Glutathione Synthesis through an ARE-Nrf2 Dependent Pathway by Zinc in the RPE. *Invest Ophthalmol Vis Sci* 2006;47:2709–2715. [PubMed: 16723490]
25. Sasaki H, Sato H, Kuriyama-Matsumura K, Sato K, Maebara K, Wang H, Tamba M, Itoh K, Yamamoto M, Bannai S. Electrophile Response Element-mediated Induction of the Cystine/Glutamate Exchange Transporter Gene Expression. *J Biol Chem* 2002;277:44765–44771. [PubMed: 12235164]
26. Sato H, Shiiya A, Kimata M, Maebara K, Tamba M, Sakakura Y, Makino N, Sugiyama F, Yagami KI, Moriguchi T, Takahashi S, Bannai S. Redox imbalance in cystine/glutamate transporter-deficient mice. *J Biol Chem* 2005;280:37423–37429. [PubMed: 16144837]
27. Moriarty-Craige SE, Adkison J, Lynn M, Gensler G, Bressler S, Jones DP, Sternberg P Jr. Antioxidant Supplementation Prevent Oxidation of Cysteine/Cystine Redox in AMD Patients. *Am J Ophthalmol* 2005;140:1020–1026. [PubMed: 16376645]
28. Grahn BH, Paterson PG, Gottschall-Pass KT, Zhang Z. Zinc and the eye. *J Am Coll Nutr* 2001;20:106–118. [PubMed: 11349933]

Biographies

Dr. Dean P. Jones is Professor of Medicine and Director of the Clinical Biomarkers Laboratory at Emory University. He received his PhD from Oregon Health Sciences University and postdoctoral training at Cornell. Dr. Jones studies mechanisms of redox circuitry in mammalian cells. He has collaborated with a number of clinical investigators, including Dr. Sternberg, on biomarker measurements in various human diseases, including age-related macular degeneration.

Dr. Paul Sternberg is the G.W. Hale Professor and Chair of the Vanderbilt Eye Institute. He is a graduate of Harvard and University Chicago and received his ophthalmology training at the Wilmer and Duke. His laboratory research interests focus on the pathogenesis of age-related macular degeneration. His team performed the ancillary studies to the AREDS, using plasma biomarkers of oxidative stress to monitor the therapeutic effects of supplementation with zinc and antioxidant vitamins.

Figure 1.

Long-term zinc supplementation resulted in lower plasma cystine (CySS) in AREDS patients. Plasma CySS was measured in AMD patients before and after 5 years of zinc supplementation. Subjects who had received zinc had significantly lower CySS than their baseline values ($p = 0.05$). Additionally, at draw 2, plasma CySS was significantly higher in the no zinc group compared to the zinc group ($p = 0.02$).

Figure 2.

Schematic diagram of a proposed mechanism by which zinc increases cellular uptake of CySS. Zinc activates the ARE/Nrf2 pathway and leads to increased expression of detoxification enzymes including the glutamate-CySS exchanger, X_c^- . Increased CySS uptake results in reduced plasma CySS concentration and potentially a number of possible downstream effects including: increased metalloproteinase activity, decreased fibronectin expression, decreased monocyte adhesion to vascular endothelial cells, and decreased cellular susceptibility to oxidant-induced apoptosis. These effects have implications in AMD. The numbers on the arrows indicate the references cited.

Table 1

Demographic Characteristics of Ancillary AREDS AMD Study Participants with Zinc and without Zinc Treatment for Blood Draw 1 and 2

Characteristic	Blood Draw 1		total n = 312	Blood Draw 2		total n = 82
	No Zinc n = 151	Zinc n = 161		No Zinc n = 41	Zinc n = 41	
Study Site (%Emory)	35.8	36	35.9	63.4	61	62.2
Sex (%female)	53.6	57.8	55.8	51.2	48.8	50
Race (%white)	96	98.1	97.1	95.1	100	97.6
Diabetes (%Yes)	9.3	8.1	8.7	9.8	2.4	6.1
High BP (%Yes)	41.7	40.4	41.0	46.3	36.6	41.5
Smoking						
Current	7.3	8.7	8.0	4.9	7.3	6.1
Former	51.0	47.8	49.3	61.0	53.7	57.3
Never	41.7	43.5	42.6	34.1	39	36.6
Mean Age at Draw	71.5	71.3	71.4	75.9	75.9	75.9
BMI	27	27.4	27.2	26.7	26.1	26.4
Time from randomization to blood draw (months)	19.2	20.7	20.0	78.6	82.4	80.0

Table 2

Metabolites and Redox Potentials from the Plasma of Ancillary AREDS AMD Study Subjects at Blood Draws 1 and 2 with and without Zinc Treatment

	Draw 1 Zinc n = 161	Draw 2 Zinc n = 41	Blood Draw p-value	Draw 1 No Zinc n = 151	Draw 2 No Zinc n = 41	p-value *
GSH (μM)	1.08	0.87	0.05	1.03	0.93	0.89
GSSG (μM)	0.09	0.08	0.06	0.08	0.10	0.001
E _h GSH (mV)	-120.8	-110.7	0.01	-122.6	-112.4	0.007
Cys (μM)	9.1	7.3	0.10	9.1	9.1	0.80
CySS (μM)	58.3	54.9	0.05	57.7	64.1	0.21
Total Cys (μM)	127.3	121.6	121.6	125.9	142.1	0.08
E _h Cys (mV)	-71.7	-68.4	0.52	-72.0	-71.6	0.99

* *p*-value for a statistical test (chi-square or Fisher's exact test for percentages, independent groups t-test for means) comparing zinc and no zinc groups at each blood draw.