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No effect of cigarette smoking dose on oxidized plasma proteins

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Abstract

Cigarette smoking is a major source of oxidative stress. Protein carbonyls have been used as a biomarker of oxidative stress because of the relative stability of carbonylated proteins and the high protein concentration in blood. Increased levels of carbonyl groups have been found in serum proteins of smokers compared to nonsmokers. However, neither the dose effect of current cigarette smoke nor other predictors of oxidative stress have been studied. Hence, we used an ELISA (Enzyme-Linked Immunosorbent Assay) to evaluate plasma protein carbonyls in smokers recruited in the Early Lung Cancer Action Project (ELCAP) program. The lung cancer screening program enrolled current and former smokers age 60 years and over without a prior cancer diagnosis. A total of 542 participants (282 men and 260 women) completed a baseline questionnaire and provided blood samples for the biomarker study. Protein oxidation was measured by derivatization of the carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) and ELISA quantitation of the DNPH group. Current smoking status was confirmed with urinary cotinine. The mean (± SD) protein carbonyl level was 17.9 ± 2.9 nmol carbonyls/ml plasma. Protein carbonyls did not differ significantly by gender. Carbonyl levels were higher among current than former smokers, but these differences did not attain statistical significance, nor did differences by urine cotinine levels, pack-years, pack/day among current smokers, and smoking duration. In a multiple regression analysis, higher protein carbonyl levels were independently associated with increasing age (0.59 nmol/ml increase per 10 years, 95% CI 0.14, 1.05, p = 0.01), African-American vs. white race/ethnicity, (1.30 nmol/ml, 95% CI 0.4, 2.19, p =0.008), and lower educational attainment (0.75 nmol/ml, 95% CI 0.12, 1.38, p = 0.02). Although we found no significant difference between current versus past cigarette smoking and protein carbonyls in this older group of smokers, associations were found for age, ethnicity and educational

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Participants at the Columbia site were consented for biomarker and genetic analyses, and the Columbia University Institutional Review Board approved all study activities.

attainment. Our results indicate that the measurement of plasma carbonyls by this ELISA technique is still an easy and suitable method for studies of diseases related to oxidative stress.

Keywords

Oxidative stress; Cigarette smoking; Protein carbonyls; Biomarker; ELCAP

1. Introduction

Cigarette smoke contains over 4000 different chemicals, 400 of which are proven carcinogens. Cigarette smoke also contains various oxidants such as oxygen free radicals (Pryor et al., 1983) and volatile aldehydes (O'Neill et al., 1994), which are probably the major causes of damage to biomolecules. Epidemiological studies have identified and randomized clinical trials have confirmed that cigarette smoking is an important risk factor for cardiovascular disease, chronic obstructive pulmonary disease, as well as for lung cancer and other malignancies (Shah & Helfant, 1988; Frank, 1993; Bartecchi et al., 1994). Oxidative stress is prominent among the hazardous effects of cigarette smoke and entails lipid peroxidation (Frei et al., 1991), protein oxidation (Reznick et al., 1992), and DNA damage (Kiyosawa et al., 1990). Increased level of 8-hydroxyguanine (8-OH-dG), a biomarker of oxidative DNA damage, in human leukocytes, lung tissues, and urine, have been observed in smokers compared to nonsmokers (Kiyosawa et al., 1990; Loft et al., 1992; Asami et al., 1996; Asami et al., 1997).

Proteins are major targets for reactive oxidants in cells and oxidized proteins that accumulate during aging, oxidative stress and in some pathological conditions (Berlett & Stadtman, 1997; Stadtman & Berlett, 1997). Exposure of proteins to reactive oxygen species results in modification of amino acid residues, which alters protein structure and function (Berlett & Stadtman, 1997). Previous studies have demonstrated that in vitro exposure of plasma to gas-phase cigarette smoke leads to the rapid accumulation of plasma protein carbonyls (Reznick et al., 1992). Increased levels of protein carbonyls have also been found in globin and serum proteins of smokers compared to nonsmokers (Lee et al., 1998; Marangon et al., 1999; Pignatelli et al., 2001).

Carbonyl groups in proteins, determined as DNPH (2,4-dinitrophenylhydrazine) derivatives, have been analyzed as a biomarker of oxidative damage of proteins (Berlett & Stadtman, 1997). In addition, a sensitive ELISA (Enzyme-Linked Immunosorbent Assay) method has been developed for measurement of protein carbonyls (Buss et al., 1997). This biomarker may be more useful than others for larger studies since proteins are present in higher concentrations in blood than is DNA, and only small amounts of blood (<50 μl) are required for analysis.

Although increased levels of protein carbonyls are found among smokers compared to nonsmokers, the dose effect of cigarette smoke on oxidative stress has not been studied. We hypothesized that smokers who currently use more cigarettes would have greater oxidative stress and have higher levels of plasma protein carbonyls, as a biomarker for oxidative stress. Hence, we used the ELISA method to evaluate plasma protein carbonyls in subjects who were recruited into a lung cancer screening cohort.

2. Materials and methods

2.1. Study population

Participants were recruits at the Columbia University site of the New York Early Lung Cancer Action Project (ELCAP)(Henschke et al., 2006). This lung cancer screening program enrolled current and former smokers with 10 or more pack-years, age 60 and over, without a prior cancer

diagnosis, who were willing to undergo screening for lung cancer with low-dose chest computed tomography (CT). The protocol specified baseline and one-year follow-up examinations, which each comprised of a low-dose CT, an interviewer-administered questionnaire, and a smoking intervention for current smokers. The Columbia University site recruited ELCAP participants into a study on biomarkers and emphysema, the EMphysema and Cancer Action Project (EMCAP). These participants completed additional questionnaires on smoking history and spirometry. Blood and urine samples were also collected in 2001-2 and stored at -80°C until analysis. Participants at the Columbia site were specifically consented for biomarker and genetic analyses, and the Columbia University Institutional Review Board approved all study activities.

Overall, 557 current and former smokers were enrolled at Columbia University. Of these, 557 (100%) completed the baseline questionnaire and CT scan, and 542 (97%) provided biological specimens.

2.2. Protein carbonyl measurement

The levels of plasma protein carbonyl groups were assessed using a noncompetitive ELISA (Buss et al., 1997), with minor modifications (Marangon et al., 1999). After determination of protein concentration (BCA-1 Protein Assay Kit, Sigma, St. Louis, MO), plasma samples were adjusted to a protein concentration of 4 mg/ml. Samples were derivatized with 2,4-dinitrophenylhydrazine (Sigma) and adsorbed to Maxisorb 96 well plates (Nunc, Life Technologies, Eggenstein, Germany). After blocking with 0.1% Tween 20, protein carbonyls were detected using a polyclonal rabbit anti-dinitrophenyl antibody (Molecular Probes Inc., 1:1500 for 1 h at 37°C) and horseradish peroxidase-conjugated secondary antibody (Amersham International, 1:4000 for 1 h at 37°C). Immunoreactivity was determined by measuring the conversion of 3,3′,5,5′-tetramethylbenzidine (TMB)(Sigma) at 450 nm after termination of the reaction with sulfuric acid. Hypochlorous acid-oxidized bovine serum albumin was used as external standard (Buss et al., 1997). Results were expressed as nmol carbonyl/ml plasma. The assay for the total carbonyl content had an interbatch coefficient of variation of 6.8% (n=16).

Cotinine levels were measured with kits from Orasure Technologies, Inc, Bethlehem, PA, as directed by the manufacturer. Smoking status was defined as current if the urinary cotinine level was ≥500 ng/mL or if the participant self-reported current smoking. Smoking status was defined as past if the participant denied current smoking and the urinary cotinine level was < 500 ng/mL. The urinary creatinine was assayed using a Creatinine kit from Sigma (St. Louis, MO; Cat. No. 555-A).

2.3. Statistical Analysis

The data on general characteristics or protein carbonyl levels were expressed as mean ± SD. The differences between strata for the general characteristics were examined by Student *t*-test and one-way analysis of variance (ANOVA) for two or more than two strata, respectively. We conducted trend tests to evaluate the dose effect of these characteristics on the level of protein carbonyls. Significant variables identified from the univariate analyses were included as covariates in multiple regression models. All analyses were performed using the SAS statistical package (version 8.2 for windows; SAS Institute, Inc., Cary, NC) and $p<0.05$ was used as the criterion for statistical significance.

3. Results

3.1. General characteristics of participants in the EMCAP Study

The general characteristics of the participants in the EMCAP study are summarized in table 1. Subjects were nearly equally divided by gender (mean age, 67 years). Among the participants,

approximately 74% were Caucasian and half attained college or advanced degrees. The mean cumulative cigarette smoke exposure was 49 pack-years, a mean years smoked of 39 years and a mean daily cigarette use of 1.1 packs among current smokers. The median urinary cotinine was much higher in current smokers than in former smokers (1772 vs. 23 ng/mL). Participants had a mean body mass index of 27.

3.2. Level of oxidized plasma proteins stratified by general characteristics

Among all participants, the mean (± SD) protein carbonyl level was 17.9 ± 2.9 nmol carbonyl/ml plasma. The levels of protein carbonyl were significant higher among participants who were older, black, and obese and significantly lower among those with advanced degrees (Table 2). We also found that females had marginally higher carbonyl content in their plasma than males (p=0.09). Although carbonyl levels were higher among current than former smokers, these differences did not attain statistical significance, nor did observed differences by pack-years, packs per day among current smokers, and smoking duration. Smoking status measured by cotinine levels did not alter the results. The middle quintile of cotinine/creatinine ratio was significantly elevated, although no linear trend across quintiles of cotinine/creatinine ratio was observed. A U-shaped relationship with BMI was observed, and obese subjects had significantly higher levels of protein carbonyl compared to normal-weight subjects.

3.3. Multivariate regression coefficients and their standard errors for oxidized protein

Multiple regression analysis showed no association between current and former cigarette smoking and protein carbonyl levels was found after adjustment for covariates (Table 3). However, higher protein carbonyl levels were independently associated with increasing age (0.59 nmol/ml increase per 10 years, 95% CI 0.14, 1.05, p=0.01), African-American vs. white race/ethnicity, (1.30 nmol/ml, 95% CI 0.4, 2.19, p=0.008), lower educational attainment (0.75 nmol/ml, 95% CI 0.12, 1.38, p=0.02) and middle quintile of cotinine/creatinine ratio (1.17 nmol/ml, 95% CI 0.40, 1.95, p=0.003). Differences in protein carbonyl levels by BMI were not statistically significant after adjustment for covariates.

4. Discussion

Although we found no association between cotinine-confirmed current and former cigarette smoking status and protein carbonyls in this older group of long-term smokers and exsmokers, associations were found for age, ethnicity and educational attainment. The results obtained indicate that the measurement of plasma carbonyls by this ELISA technique is still relatively easy and suitable for large studies of oxidative stress-related disease.

Previous studies have found that smokers have higher contents of protein carbonyls in globin and serum proteins than nonsmokers (Lee et al., 1998; Marangon et al., 1999; Pignatelli et al., 2001). Using the colorimetric assay, Lee et al. examined the chemopreventive effect of antioxidants on cigarette smoke-induced oxidative stress among 15 smokers and 5 nonsmokers (Lee et al., 1998). They found a significantly elevated carbonyl level in globin from smokers compared to nonsmokers (2.56 vs. 1.59 nmol/mg protein, p<0.01). A study using the ELISA method showed that smokers have higher plasma protein carbonyls than individually matched nonsmokers (N=22) (Marangon et al., 1999). The levels of oxidized serum proteins measured by western-blot assay were also significantly associated with smoking status, whereas no difference was seen between heavy and light smokers (Pignatelli et al., 2001). The protein carbonyl level among the older and heavier smokers in the present study (17.9 nmol carbonyl/ml plasma) was comparable to our breast cancer study (28.3% controls were age > 65 years) using the same ELISA method (under revision). Although no significant association was seen between protein carbonyl levels and cigarette smoking status, we did find smokers have higher carbonyl levels than nonsmokers (protein carbonyls among health controls were 16.6, 16.2 and

15.7 nmol carbonyl/ml plasma for current, former and never smokers, respectively). Because the participants enrolled in our study were current and former smokers, we could not explore the difference between smokers and nonsmokers. Nevertheless, our findings are in agreement with the latest report and our breast cancer study that found no significant dose effect of smoking on protein carbonyls (Pignatelli et al., 2001).

We found the protein carbonyl levels were significantly higher among those who were elderly, African-American, less well educated and obese. Oxidative modifications of intracellular proteins that accrue during aging have been suggested to play a key role in the causation of senescence-associated losses in physiological functions (Stadtman, 1992; Berlett & Stadtman, 1997; Stadtman & Berlett, 1997; Stadtman, 2001). Addition of carbonyl-containing adducts to the side chains of amino acid residues is the most well characterized, age-associated, post-translational structural alteration in proteins (Stadtman, 1992; Berlett & Stadtman, 1997; Stadtman & Berlett, 1997; Stadtman, 2001). The results in the present study of a positive association between plasma protein carbonyl level and increasing age supports the results cited in these review articles.

Oxidative stress is different among ethnic groups. Numerous studies have reported that biomarkers for oxidative stress, including plasma F2-isoprostanes (Lopes et al., 2003), serum C-reactive protein (Lee & Jacobs, 2005) and coenzyme Q10 (Miles et al., 2003) were greater in African Americans than in whites. In addition, the prevalence of diseases related to oxidative stress-related endothelial dysfunction, such as hypertension and diabetes mellitus, is considerably greater in blacks than whites (Kalinowski et al., 2004). Whether these differences are due to social or biological factor is not clear. We also found that educational attainment, as an inexact measure of socioeconomic status (SES), was significantly related to plasma protein carbonyl level. Our results suggest that SES may be an explanation for the observed differences by race/ethnicity and educational level.

Protein carbonyl level was significantly elevated in subjects in the middle quintile of the cotinine/creatinine ratio, although no dose response trend across quintiles was observed. Without a plausible biologic mechanism, this result may be due to chance. We also observed that plasma protein carbonyl concentrations were elevated among obese subjects, although the differences were not statistically significant in multivariate analysis. Oxidative stress is considered to be one of the main causes of molecular damage to cellular and tissue structures and is known to be increased in patients with diabetes mellitus (Baynes, 1991; Giugliano et al., 1996). Studies have also provided evidence that obesity may be associated with defective antioxidant status and enhanced lipid peroxidation (Davi et al., 2002; Ozata et al., 2002). Because carbonyl content is a good biomarker for oxidative stress, our finding is reasonable. A recent study has reported an increase in reactive oxygen species-induced damage in lipids, proteins, and amino acids in the obese compared with normal subjects (Dandona et al., 2001). They also found that plasma protein carbonyl levels were reduced after 4 weeks of caloric restriction.

Increased oxidative stress in smokers has been shown by measuring various biomarkers, including lipid peroxidation products in plasma (Reilly et al., 1996; Miller et al., 1997), oxidized DNA bases in leukocyte DNA and urine (Kiyosawa et al., 1990; Asami et al., 1996; van Zeeland et al., 1999) and F2-isoprostanes in plasma and urine (Morrow et al., 1995; Reilly et al., 1996). It would be worthwhile to further study the correlation between these biomarkers and levels of oxidized proteins in relation to smoking habits.

This study has a number of strengths, including relatively large size, precise measurement of protein carbonyls, and confirmation of current smoking status by urinary cotinine. Limitations included lack of a nonsmoking control group. We therefore could not determine if protein

carbonyls were higher among ever smokers than never smokers, although the mean values in both current and former smokers suggest this to be the case compared to our breast cancer study and previously published studies of never smokers (Pignatelli et al., 2001). The cross-sectional design meant that we could not rule out reverse causality, and it is possible that smokers who were particularly susceptible to smoking and had elevated levels of protein carbonyls quit smoking before enrollment in the study. The health status and nutritional information of the participants were not assessed, which are likely to bias our results. Elevated protein oxidation have been associated with neurological and inflammatory disease, and some antioxidants may decrease oxidative stress (Mayne, 2003). However, we found no association of protein carbonyl with chronic obstructive pulmonary disease (COPD) and the adjustment for information on antioxidant supplementation use for a subgroup (n=134) of our participants also did not alter results (data not show). Finally, protein carbonyls were measured on specimens stored for several years at -80°C; it is not certain whether protein oxidized during storage. However, one study measuring protein carbonyl in saliva showed that neither fresh or frozen conditions altered the carbonyl values (Nagler et al., 2000).

Although proteins are major targets for oxidative damage in vivo (Berlett & Stadtman, 1997; Davies et al., 1999), the modified proteins in human plasma have not been extensively measured as possible biomarkers of oxidative stress in relation to human nutrition, disease status, and life-style. The ELISA method described in this study is sensitive and specific for oxidized proteins (Dalle-Donne et al., 2003). In addition, this marker may be more useful than others for larger studies since proteins are present in higher concentrations in blood than is DNA, small amounts of plasma (<20 μl) are required for analysis, and oxidized proteins are relatively stable, allowing for its more sensitive detection. Studies to investigate the effects of antioxidants and interaction between cigarette smoking and protein carbonyls on disease risk are warranted.

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References

Asami S, Hirano T, Yamaguchi R, Tomioka Y, Itoh H, Kasai H. Increase of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in human leukocytes by cigarette smoking. Cancer Res 1996;56:2546–2549. [PubMed: 8653695]

Asami S, Manabe H, Miyake J, Tsurudome Y, Hirano T, Yamaguchi R, Itoh H, Kasai H. Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung. Carcinogenesis 1997;18:1763–1766. [PubMed: 9328173]

Bartecchi CE, MacKenzie TD, Schrier RW. The human costs of tobacco use (1). N. Engl. J. Med 1994;330:907–912. [PubMed: 8114863]

Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40:405–412. [PubMed: 2010041]

Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. J. Biol. Chem 1997;272:20313–20316. [PubMed: 9252331]

Buss H, Chan TP, Sluis KB, Domigan NM, Winterbourn CC. Protein carbonyl measurement by a sensitive ELISA method. Free Radic. Biol. Med 1997;23:361–366. [PubMed: 9214571]

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]

Dandona P, Mohanty P, Ghanim H, Aljada A, Browne R, Hamouda W, Prabhala A, Afzal A, Garg R. The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. J. Clin. Endocrinol. Metab 2001;86:355–362. [PubMed: 11232024]

Davi G, Guagnano MT, Ciabattoni G, Basili S, Falco A, Marinopiccoli M, Nutini M, Sensi S, Patrono C. Platelet activation in obese women: role of inflammation and oxidant stress. JAMA 2002;288:2008–2014. [PubMed: 12387653]

Davies MJ, Fu S, Wang H, Dean RT. Stable markers of oxidant damage to proteins and their application in the study of human disease. Free Radic. Biol. Med 1999;27:1151–1163. [PubMed: 10641706]

Frank E. Benefits of stopping smoking. West. J. Med 1993;159:83–86. [PubMed: 8351918]

Frei B, Forte TM, Ames BN, Cross CE. Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. Biochem. J 1991;277(Pt 1):133–138. [PubMed: 1854329]

Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996;19:257–267. [PubMed: 8742574]

Henschke CI, Yankelevitz DF, Libby DM, Pasmantier MW, Smith JP, Miettinen OS. Survival of patients with stage I lung cancer detected on CT screening. N. Engl. J. Med 2006;355:1763–1771. [PubMed: 17065637]

Kalinowski L, Dobrucki IT, Malinski T. Race-specific differences in endothelial function: predisposition of African Americans to vascular diseases. Circulation 2004;109:2511–2517. [PubMed: 15159296]

Kiyosawa H, Suko M, Okudaira H, Murata K, Miyamoto T, Chung MH, Kasai H, Nishimura S. Cigarette smoking induces formation of 8-hydroxydeoxyguanosine, one of the oxidative DNA damages in human peripheral leukocytes. Free Radic. Res. Commun 1990;11:23–27. [PubMed: 2074046]

Lee BM, Lee SK, Kim HS. Inhibition of oxidative DNA damage, 8-OHdG, and carbonyl contents in smokers treated with antioxidants (vitamin E, vitamin C, beta-carotene and red ginseng). Cancer Lett 1998;132:219–227. [PubMed: 10397477]

Lee DH, Jacobs DR Jr. Association between serum gamma-glutamyltransferase and C-reactive protein. Atherosclerosis 2005;178:327–330. [PubMed: 15694941]

Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis 1992;13:2241–2247. [PubMed: 1473230]

Lopes HF, Morrow JD, Stojiljkovic MP, Goodfriend TL, Egan BM. Acute hyperlipidemia increases oxidative stress more in African Americans than in white Americans. Am. J. Hypertens 2003;16:331–336. [PubMed: 12745192]

Marangon K, Devaraj S, Jialal I. Measurement of protein carbonyls in plasma of smokers and in oxidized LDL by an ELISA. Clin. Chem 1999;45:577–578. [PubMed: 10102923]

Mayne ST. Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J. Nutr 2003;133(Suppl 3):933S–940S. [PubMed: 12612179]

Miles MV, Horn PS, Morrison JA, Tang PH, DeGrauw T, Pesce AJ. Plasma coenzyme Q10 reference intervals, but not redox status, are affected by gender and race in self-reported healthy adults. Clin. Chim. Acta 2003;332:123–132. [PubMed: 12763289]

Miller ER 3rd, Appel LJ, Jiang L, Risby TH. Association between cigarette smoking and lipid peroxidation in a controlled feeding study. Circulation 1997;96:1097–1101. [PubMed: 9286935]

Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ 2nd. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N. Engl. J. Med 1995;332:1198–1203. [PubMed: 7700313]

Nagler R, Lischinsky S, Diamond E, Drigues N, Klein I, Reznick AZ. Effect of cigarette smoke on salivary proteins and enzyme activities. Arch. Biochem. Biophys 2000;379:229–236. [PubMed: 10898939]

O'Neill CA, Halliwell B, van der Vliet A, Davis PA, Packer L, Tritschler H, Strohman WJ, Rieland T, Cross CE, Reznick AZ. Aldehyde-induced protein modifications in human plasma: protection by glutathione and dihydrolipoic acid. J. Lab. Clin. Med 1994;124:359–370. [PubMed: 8083579]

Ozata M, Mergen M, Oktenli C, Aydin A, Sanisoglu SY, Bolu E, Yilmaz MI, Sayal A, Isimer A, Ozdemir IC. Increased oxidative stress and hypozincemia in male obesity. Clin. Biochem 2002;35:627–631. [PubMed: 12498997]

Pignatelli B, Li CQ, Boffetta P, Chen Q, Ahrens W, Nyberg F, Mukeria A, Bruske-Hohlfeld I, Fortes C, Constantinescu V, Ischiropoulos H, Ohshima H. Nitrated and oxidized plasma proteins in smokers and lung cancer patients. Cancer Res 2001;61:778–784. [PubMed: 11212282]

Pryor WA, Prier DG, Church DF. Electron-spin resonance study of mainstream and sidestream cigarette smoke: nature of the free radicals in gas-phase smoke and in cigarette tar. Environ. Health Perspect 1983;47:345–355. [PubMed: 6297881]

Reilly M, Delanty N, Lawson JA, FitzGerald GA. Modulation of oxidant stress in vivo in chronic cigarette smokers. Circulation 1996;94:19–25. [PubMed: 8964113]

Reznick AZ, Cross CE, Hu ML, Suzuki YJ, Khwaja S, Safadi A, Motchnik PA, Packer L, Halliwell B. Modification of plasma proteins by cigarette smoke as measured by protein carbonyl formation. Biochem. J 1992;286(Pt 2):607–611. [PubMed: 1530591]

Shah PK, Helfant RH. Smoking and coronary artery disease. Chest 1988;94:449–452. [PubMed: 3409719]

Stadtman ER. Protein oxidation and aging. Science 1992;257:1220–1224. [PubMed: 1355616]

Stadtman ER. Protein oxidation in aging and age-related diseases. Ann. N. Y. Acad. Sci 2001;928:22–38. [PubMed: 11795513]

Stadtman ER, Berlett BS. Reactive oxygen-mediated protein oxidation in aging and disease. Chem. Res. Toxicol 1997;10:485–494. [PubMed: 9168245]

van Zeeland AA, de Groot AJ, Hall J, Donato F. 8-Hydroxydeoxyguanosine in DNA from leukocytes of healthy adults: relationship with cigarette smoking, environmental tobacco smoke, alcohol and coffee consumption. Mutat. Res 1999;439:249–257. [PubMed: 10023075]

Table 1

General characteristics of participants in the EM CAP Study at baseline

Characteristic	N=541	%	Mean(SD)
Sex			
Male	281	52	
Female	260	48	
Mean age	541		67 (5)
Ethnicity			
White	398	74	
Hispanic	49	9	
Black	42	8	
Asian/Pacific	52	10	
Educational Attainment			
High school degree	250	46	
College degree	148	27	
Graduate degree	143	26	
Smoking History			
Former	317	59	
Current	224	41	
Mean pack-years	541		49 (26)
Mean years smoked	541		39 (12)
Mean packs/day among current smokers	541		1.1 (0.6)
Median urine cotinine (ng/ml)(IQR)			
Former smokers	317		23 (45)
Current smokers	224		1772(1048)
Mean BMI	541		27 (5)

Table 2

Level of oxidized plasma proteins stratified by general characteristics

Characteristic	n	nmol carbonyIs/ml plasma (mean ± SD)	Age-adjusted beta (95%CI)	p-value
Sex				
Male	281	17.7 ± 2.8	Ref	
Female	260	18.1 ± 3.0	0.417 (-0.071, 0.905)	0.094
Age range (years)				
59, <65	245	17.7 ± 2.9	Ref	
65, <70	147	17.8 ± 3.1	0.065 (-0.529, 0.658)	0.83
>70	149	18.3 ± 2.7	0.623 (0.032, 1.215)	0.039
p for trend				0.012
Ethnicity				
White	398	17.8 ± 2.9	Ref	
Hispanic	49	17.3 ± 2.9	-0.428 (-1.279, 0.423)	0.32
Black	42	19.5 ± 2.8	1.645 (0.731,2.558)	0.0004
Asian	52	17.9 ± 2.8	0.061 (-0.770, 0.891)	0.89
Educational Attain				
High School	250	18.1 ± 2.7	Ref	
College Degree	148	18.0 ± 3.1	-0.167 (-0.753, 0.419)	0.58
Graduate Degree	143	17.4 ± 3.0	-0.759 (-1.352, -0.166)	0.012
p for trend				0.076
Smoking Status				
Former	317	17.9 ± 2.9	Ref	
Current	224	17.9± 2.9	0.155 (-0.345, 0.655)	0.54
Pack-years				
<30	132	18.0 ± 2.9	Ref	
>30 <50	185	17.7 ± 2.9	-0.209 (-0.858, 0.441)	0.53
>50	224	18.0 ± 2.9	0.014 (-0.610, 0.638)	0.97
p for trend				0.99
Years smoked (years)				
<30	113	17.7 ± 2.7	Ref	
30, <45	221	17.9 ± 2.8	0.374 (-0.288, 1.037)	0.27
>45	207	17.9 ± 3.1	0.181 (-0.486, 0.849)	0.59
p for trend				0.39
Current packs per day				
0	317	17.8 ± 2.9	Ref	
<1	59	18.3 ± 2.6	0.492 (-0.313, 1.30)	0.23
1, <1.5	94	18.1 ± 3.1	0.313 (-0.356, 0.982)	0.36
>1.5	62	17.6 ± 2.8	-0.148 (-0.943, 0.647)	0.72
p for trend				0.88
Urine cotinine level (ng/mL)				
<100	289	17.8 ± 2.9	Ref	
100, <500	41	18.6 ± 3.6	0.762 (-0.188, 1.711)	0.116
≥500	201	17.9± 2.8	0.142 (-0.384, 0.667)	0.597
p for trend				0.82
Cotinine/creatinine ratio				
<14	107	17.4 ± 2.8	Ref	
>14, <40	101	17.7 ± 2.8	0.257 (-0.527, 1.041)	0.520
>40, <300	108	18.6± 3.0	1.170 (0.400, 1.938)	0.003
>300, <1500	103	18.2± 3.3	0.817 (0.038, 1.596)	0.040
>1500	111	17.5± 2.6	0.170 (-0.595, 0.934)	0.663
p for trend				0.56
BMI (kg/m^2)				
<20	32	18.3 ± 3.7	0.678 (-0.405, 1.761)	0.22
20, <25	192	17.6 ± 2.8	Ref	
25-30	196	17.9 ± 2.9	0.338 (-0.239, 0.916)	0.25
≥30	121	18.2 ± 2.8	0.717 (0.053, 1.381)	0.034
p for trend				0.087

Table 3

Multivariate regression coefficients (in nmole carbonyls/ml plasma) and their standard errors (S.E.) for oxidized protein*

Characteristic	β**	S.E.	p-value
Intercept	13.290	1.635	<0.0001
Age (per 10-year increase)	0.59	0.23	0.010
Sex			
Female vs. Male	0.260	0.266	0.328
Ethnicity			
Hispanic vs. White	-0.583	0.456	0.217
Black vs. White	1.299	0.491	0.008
Asian vs. White	0.241	0.460	0.600
Educational Attainment			
College vs High School Degree	-0.028	0.312	0.929
Graduate vs High School Degree	-0.751	0.321	0.020
p-trend for education			0.089
Smoking status (current vs former)	0.730	0.578	0.207
Years smoked (per 10 years)	0.052	0.11	0.637
Self-reported packs per day	-0.0971	0.196	0.621
Urine cotinine level	-0.00008	0.0001	0.488
Packyears	-0.001	0.005	0.767
Cotinine Creatinine Ratio (CCR)			
14<CCR<40 vs 0<CCR<14	0.241	0.399	0.546
40<CCR<300 vs 0<CCR<14	1.174	0.395	0.003
300<CCR<1500 vs 0<CCR<14	-0.005	0.631	0.994
1500<CCR vs O<CCR<14	-0.581	0.672	0.388
BMI (kg/m^2)			
0<BMI<20 vs 20≤BMI<25	0.312	0.561	0.578
25≤BMI<30 vs 20≤BMI<25	0.257	0.297	0.388
30≤BMI vs 20≤BMI<25	0.557	0.3456	0.108
p-trend for BMI			0.155

* Adjusted for variables in the table.

** β is change in plasma carbonyl (nmole/ml plasma) per unit change in characteristic.