Biomarkers, June 2008; 13(4): 422–434

informa
healthcare

Plasma carbonyls do not correlate with lung function or computed tomography measures of lung density in older smokers

SONIA MESIA-VELA[1], CHIH-CHING YEH[2,3], JOHN H.M. AUSTIN[4], MATTHEW DOUNEL[4], CHARLES A. POWELL[1], ANTHONY REEVES[5], REGINA M. SANTELLA[3], LORI STEVENSON[1], DAVID YANKELEVITZ[6], & R. GRAHAM BARR[1,7]

[1]*Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY, USA,* [2]*Department of Health Risk Management, China Medical University College of Public Health, Taichung, Taiwan,* [3]*Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY, USA,* [4]*Department of Radiology, College of Physicians and Surgeons, Columbia University, New York, NY, USA,* [5]*Department of Engineering, Cornell University, Ithaca, NY, USA,* [6]*Department of Radiology, Cornell University, New York, NY, USA and* [7]*Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, USA*

Abstract

Oxidative stress and inflammation are hallmarks of chronic obstructive pulmonary disease (COPD). A critical byproduct of oxidative damage is the introduction of carbonyl groups into amino acid residues. We hypothesize that plasma carbonyl content is inversely correlated with lung function and computed tomography (CT) measures of lung density among smokers and is elevated in COPD. Carbonyl was measured in plasma of participants aged 60 years and older by ELISA. Generalized linear and additive models were used to adjust for potential confounders. Among 541 participants (52% male, mean age 67 years, 41% current smokers), mean plasma carbonyl content was 17.9 ± 2.9 nmol ml^{-1} and mean forced expiratory volume in one second (FEV_1) was 80.7 ± 20.9% of predicted. Plasma carbonyl content was inversely associated with FEV_1, but this relationship was largely explained by age. Multivariate analyses ruled out clinically meaningful associations of plasma carbonyl content with FEV_1, FEV_1/FVC (forced vital capacity) ratio, severity of airflow obstruction, and CT lung density. Plasma carbonyl content is a poor biomarker of oxidative stress in COPD and emphysema.

Keywords: *Oxidative stress, smoking, protein carbonyl, EMCAP, COPD, airflow obstruction, pulmonary emphysema, CT*

(Received 3 August 2007; accepted 19 November 2007)

Correspondence: R. Graham Barr, Department of Medicine, College of Physicians and Surgeons, Columbia University Medical Center, 630 West 168th Street, PH9E Rm 105B, New York, NY 10032, USA. Fax: +1-212-305-9349. E-mail: rgb9@columbia.edu

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500802002859

Introduction

The high incidence, morbidity, and mortality rates of chronic obstructive pulmonary disease (COPD) make this disease the fourth highest cause of death in the United States and other countries (Snider 1985). COPD is a chronic and progressive inflammatory disease characterized by a slow development of an irreversible airflow limitation (Barnes 2004). The increased concentration of free radicals and reactive oxygen species (ROS) found in the lungs of COPD patients (Halliwell 1996) indicates that oxidative stress may play a major role in the pathogenesis of the disease. Cigarette smoke is the major cause of COPD in part due to the large variety of oxidants in cigarette smoke which are capable of reacting quickly with different anions and oxygen to create free radicals (Church & Pryor 1985, Pryor & Stone 1993).

In the lungs, ROS are implicated in the remodelling of extracellular matrix and blood vessels, stimulation of mucus secretion, inactivation of antiproteases, induction of apoptosis, regulation of cell proliferation, gene expression of proinflammatory molecules, and the worsening of airspace inflammation (Rahman et al. 1996, Rahman & MacNee 1999). These observations suggest that inflammation and oxidative stress are co-dependent and strongly inter-related processes.

Proteins are major targets for reactive oxidants in cells. Oxidized proteins accumulate during aging, oxidative stress, and in some pathological conditions, including COPD (Stadtman & Berlett 1997, Berlett & Stadtman 1997). Introduction of carbonyl groups into amino acid residues is the most general byproduct of oxidative damage of proteins, among a wide variety of protein oxidations, and quantification of carbonyl content is generally used to estimate total protein oxidation.

Previous studies have demonstrated that *in vitro* exposure of plasma to gas-phase cigarette smoke leads to a rapid accumulation of plasma protein carbonyl content (Reznick et al. 1992). Increased levels of protein carbonyls have also been found in the globin and serum proteins of smokers compared with non-smokers (Lee et al. 1998, Marangon et al. 1999a, Pignatelli et al. 2001). Nadeem et al. (2005) found protein carbonyls to be elevated in patients with COPD compared with non-smoking controls; however, it is unclear if this elevation was due to the disease process in COPD or simply due to smoking. We therefore evaluated if plasma carbonyl content may constitute a biomarker of oxidative stress in COPD, hypothesizing that protein carbonyl content would be inversely associated with spirometric measures of lung function and computed tomography (CT) measures of lung density among older smokers.

Materials and methods

Study population and design

We recruited smokers who enrolled into a lung cancer screening program, the New York Early Lung Cancer Action Project (ELCAP) (Henschke et al. 2006). Inclusion criteria were 10 or more pack years, age 60 and over and willingness to undergo baseline and 1-year follow-up screening for lung cancer with low-dose chest CT. Individuals with a history of cancer (other than non-melanoma skin cancer) were excluded.

All New York ELCAP participants at the Columbia site were asked in 2001–02 to enrol into a study on COPD and emphysema, the EMphysema and Cancer Action

Project (EMCAP). In addition to undergoing baseline CT scans and, for current smokers, a smoking intervention, EMCAP participants underwent spirometric testing, completed an additional questionnaire on smoking history and symptoms, and provided blood and urine samples. Biospecimens were processed within 2 h of collection and aliquoted before storage at −70°C until analysis. Participants at the Columbia site were specifically consented for biomarker analyses for COPD, and the Columbia University Institutional Review Board approved all study activities.

Of 557 current and former smokers enrolled into the ELCAP lung cancer screening program at Columbia University, 100% completed the baseline questionnaire and CT scan, 522 (94%) completed adequate spirometry, and 542 (97%) provided biological specimens. The current analysis was restricted to 541 participants with complete information on plasma carbonyl content, among whom 510 (94%) had adequate spirometry, 506 (94%) had available measures of CT % emphysema and 496 (92%) had available measures of CT alpha.

Protein carbonyl measurement

The levels of plasma protein carbonyl groups were assessed using a non-competitive ELISA (Buss et al. 1997), with minor modifications (Marangon et al. 1999a). After determination of protein concentration (BCA-1 Protein Assay Kit, Sigma, St Louis, MO, USA), plasma samples were adjusted to a protein concentration of 4 mg ml^{-1}. 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^{-1} plasma. The assay for the total carbonyl content had an interbatch variation of 6.8% ($n = 16$).

Urinary cotinine measurement

Samples were analyzed for cotinine using an ELISA kit (Orasure Technologies, Inc, Bethlehem, PA, USA). The results were expressed in terms of nanograms per millilitre adjusted for creatinine levels. A 5% quality control, inter- and intraplate was performed. All samples, calibrators and controls were run in duplicates. Concentration of calibrator cotinine used for the standard curve was in the range of 0–250 ng ml^{-1}.

Lung function

Spirometry was performed following and meeting ATS guidelines (American Thoracic Society 1995) using the portable ndd EasyOne Diagnostic spirometer (ndd Medical Technologies, Chelmsford, MA, USA). We previously validated use of this spirometer for this cohort against a dry seal, rolling-barrel spirometer (Barr et al. 2008). Airflow obstruction was defined by a pre-bronchodilator FEV_1/FVC (forced

expiratory volume in one second/forced vital capacity) ratio <0.70, and severity was defined following ATS and ERS (European Respiratory Society) thresholds for COPD (Celli & MacNee 2004) based on pre-bronchodilator measures ($>80\%$ predicted, mild; 50–80% predicted, moderate and $<50\%$ predicted, severe).

Lung density measures

All participants underwent low-dose, non-contrast, full-lung CT scanning on a GE Lightspeed 4 scanner (140 kVp, 40 mA, with 6:1 pitch, 1.25 mm slice thickness, single- breathhold, with contiguous slices from the thoracic inlet to the adrenal glands). Two measures of lung density, % emphysema and alpha, were calculated from the CT images.

The percentage of emphysema present in the lungs was assessed from CT scans as the proportion of lung volume below a threshold attenuation of -910 Hounsfield units (HU) compared to the total volume of the lung. This measure is also commonly referred to as the emphysema index or density mask (Muller et al. 1988). In this study, we modified the base threshold to correct for interscan variations using the attenuation of tracheal air. We first determined the average attenuation of a cylindrical sample of air in the trachea taken above the bronchial branch point. The attenuation of the sampled air was then compared with an average attenuation of -970 HU, with the difference used to correct the baseline index attenuation of -910 HU.

We also computed the average fractal dimension of three slices as described by Mishima et al. (1999) in which the cumulative distribution of low attenuation area (LAA) sizes is fit to a power law, giving the fractal dimension D. Stated in another way, D represents the slope of the log–log plot of LAA size versus percentage of LAA on the CT image. In order to account for all emphysema, we used a threshold of -910 HU to select the LAAs and sampled the transverse slices marking 20%, 40%, and 60% of the distance craniocaudally between the apices and bases of the lungs. This method eliminates the noise of the upper- and lower-most regions of the lungs and is a robust method for calculating the fractal dimension of the lungs. The fractal dimension D was multiplied to -1 to yield the measure alpha, as previously described (Hoffman et al. 2006).

Covariate data

At the time of enrolment, information on sex, ethnicity, age, educational level, smoking history, and pack years were recorded on a questionnaire during an interview. We also collected information on history of physician-diagnosed asthma and COPD, age of onset of symptoms, and types of medications used for COPD. Smoking status was defined as current if participant self-reported current smoking and urinary cotinine level was consistent with active smoking (≥ 500 ng ml^{-1}) (Zielinska-Danch et al. 2007). Otherwise, participant was classified as past smoker (<500 ng ml^{-1}).

Statistical analysis

The data on general characteristics or protein carbonyl content were expressed as mean $\pm$ SD. The differences between strata for the general characteristics were examined by Student's *t*-test and one-way analysis of variance (ANOVA) for two or more than two strata, respectively. For the main analyses, protein carbonyl content

was divided into quintiles. Lung function and lung density measures were compared across quintiles of protein carbonyl content, and linear regression was used to adjust lung function and lung density measures for covariates, including age, sex, race/ethnicity, and height, in addition to the potential confounders of educational attainment, smoking status, pack years, body mass index (BMI) and history of asthma. This strategy was employed, rather than use of per cent of predicted FEV_1 and FVC, due to the sizable minority of this cohort (US Hispanics of non-Mexican origin and Asian/Pacific Islanders) for whom accurate prediction equations are currently unavailable. Results were confirmed and the possibility of non-linear relationships examined using generalized additive models with flexible, non-linear smoothing functions for protein carbonyl content and adjusting for the same covariates. Analyses were performed using the SAS statistical package (9.1 for Windows; SAS Institute, Inc., Cary, NC, USA) and Splus (6.2 for Windows, Insightful Corp, Seattle, WA, USA). Two-tailed $p < 0.05$ was used as the criteria for statistical significance.

Results

The characteristics of the participants in the EMCAP study with plasma carbonyl levels are summarized in Table I. The mean age was 67 years, 52% were male, 26% were non-white, and 41% were current smokers. Mean cumulative exposure to cigarette smoke was 49 pack-years. Although 17% reported a physician diagnosis of COPD, 48% had evidence of airway obstruction defined as pre-bronchodilator $FEV_1/FVC < 0.70$. Of these, 27% had mild disease, 56% had moderate and 17% severe disease. There were no significant differences with respect to sex, race/ethnicity, smoking status and pack-years between participants with complete information compared with those with missing information on plasma carbonyl content, spirometry and lung density, although the mean age was 67 years among participants with complete information compared with 70 years among those excluded due to missing information.

The overall mean plasma carbonyl content was 17.9 ± 2.9 nmol carbonyl ml^{-1}, and mean plasma carbonyl content ranged from a mean of 14.1 nmol ml^{-1} in the lowest quintile to 22.2 nmol ml^{-1} in the highest quintile. Plasma carbonyl content was inversely associated with FEV_1 (p-trend $= 0.04$) in unadjusted analyses; however, adjustment for sex and particularly age rendered this association null (Table II). Multivariate adjustment further attenuated the association. To examine the association of plasma carbonyl content and FEV_1 using a more statistically flexible methodology, we plotted the multivariate difference from mean FEV_1 by plasma carbonyl content, treating both as continuous variables. Figure 1A shows a convincingly null multivariate relationship, with tight 95% confidence interval (CI) and no suggestion of non-linearity.

Similar results were observed for FVC (Table II and Figure 1B), although the unadjusted association between plasma carbonyl content and FVC was not quite statistically significant. No associations were observed between plasma protein carbonyl content and FEV_1/FVC ratio in unadjusted or multivariate results (Table II). Associations were similar in analysis stratified by smoking status and there was no interaction between this association and smoking status (p for interaction for $FEV_1 = 0.61$). When analyses were repeated with a threshold for current smoking of

Table I. Characteristics of participants in the EMCAP study with known plasma protein carbonyl content.

	$n=541$
Age (years), mean ± SD	67 ± 6
Gender – female (%)	48
Ethnicity (%)	
White	74
Hispanic	9
Black	8
Asian/Pacific Islander	10
Highest educational attainment (%)	
No high school degree	8
High school degree	38
College degree	27
Graduate degree	26
Smoking status (%)	
Former	59
Current	41
Pack-years, mean ± SD	49 ± 26
Duration of smoking, mean ± SD	39 ± 12
Packs per day among current smokers, mean ± SD	1.14 ± 0.55
BMI, mean ± SD	27 ± 5
Physician diagnosis of COPD (%)	17
Physician diagnosis of asthma before age 45 (%)	7
Use of COPD medication in past year (%)	
Short-acting β-agonists	9
Long-acting β-agonists	5
Anticholinergic inhalers	3
Theophylline	1
Inhaled steroids	8
Systemic steroids or corticosteroids? (Austin)	3
Antibiotics	6
Home oxygen	0.2
Pre-bronchodilator:	
FVC, mean ± SD	3.22 ± 0.90
FVC, % predicted, mean ± SD	88.5 ± 19.0
FEV_1, mean ± SD	2.22 ± 0.72
FEV1, % predicted, mean ± SD	80.7 ± 20.9
FEV_1/FVC ratio, mean ± SD	0.69 ± 0.10
CT % emphysema, mean ± SD	27.19 ± 16.12
CT alpha, mean ± SD	1.22 ± 0.35

50 ng ml^{-1}, results were unchanged (data not shown). No significant associations were observed for air flow obstruction or severity of airflow obstruction; effect estimates were close to the null (Table III).

The mean % of emphysema was 27% and the CT alpha was 1.22. Higher carbonyl levels tended to an inverse association with CT % emphysema and more with CT alpha (Figure 1C, D) but no statistical significance was attained. Table IV shows the CT lung density measures, % emphysema and alpha, stratified by quintile of plasma carbonyl content. There were no significant associations between carbonyl content and CT % emphysema or CT alpha unadjusted, age- or sex-adjusted models, or

Table II. Lung function in current and former smokers, according to quintiles of plasma carbonyl content.

	Quintile of plasma carbonyl content					
	Q.1 ($n=103$)	Q.2 ($n=106$)	Q.3 ($n=102$)	Q.4 ($n=99$)	Q.5 ($n=100$)	p for trend
Plasma carbonyl content (mean ± SD)	14.1 ± 1.2	16.3 ± 0.4	17.6 ± 0.4	19.2 ± 0.6	22.2 ± 1.5	–
FEV_1 (l)	2.34	2.20	2.22	2.20	2.15	0.04
Difference in FEV_1 (l)	0	−0.11	−0.06	−0.12	0.07	0.33
(95% CI)[a]	(ref)	(−0.27, 0.06)	(−0.23, 0.10)	(−0.29, 0.05)	(−0.23, 0.10)	
Difference in FEV_1 (l) (95% CI)[b]	0 (ref)	−0.07 (−0.22, 0.08)	−0.02 (−0.17, 0.13)	−0.07 (−0.23, 0.08)	−0.01 (−0.17, 0.14)	0.59
FVC (l)	3.33	3.22	3.22	3.17	3.14	0.06
Difference in FVC (l) (95% CI)[a]	0 (ref)	−0.09 (−0.28, 0.11)	−0.06 (−0.25, 0.14)	−0.16 (−0.36, 0.04)	−0.04 (−0.24, 0.16)	0.41
Difference in FVC (l) (95% CI)[b]	0 (ref)	−0.02 (−0.20, 0.15)	0.03 (−0.15, 0.21)	−0.07 (−0.25, 0.11)	0.05 (−0.13, 0.23)	0.94
FEV_1/FVC ratio	0.70	0.68	0.69	0.69	0.68	0.38
Difference in FEV_1/FVC (95% CI)[a]	0 (ref)	−0.02 (−0.05, 0.01)	−0.01 (−0.04, 0.02)	−0.01 (−0.04, 0.02)	−0.01 (−0.04, 0.02)	0.63
Difference in FEV_1/FVC (95% CI)[b]	0 (ref)	−0.02 (−0.05, 0.003)	−0.01 (−0.04, 0.02)	−0.01 (−0.04, 0.02)	−0.01 (−0.04, 0.01)	0.50

[a]Differences adjusted for age and sex.

[b]Differences adjusted for age, sex, height, race/ethnicity, educational attainment, smoking status, pack-years, urinary cotinine level, body mass index, and physician diagnosis of asthma before age 45 years.

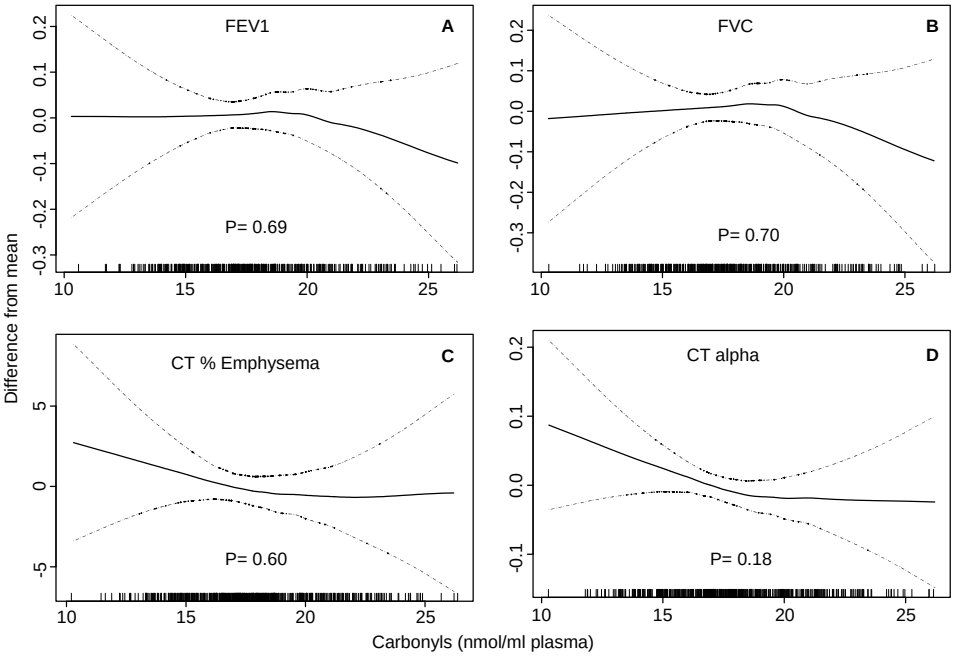

Figure 1. Oxidized plasma protein, lung function and emphysema. Lung function was assessed in function of FEV_1 (A) and FVC (B) and lung density by CT % emphysema (C) and CT alpha (D). Solid lines, smoothed regression lines adjusted for sex, age, race/ethnicity, education, smoking status, pack years and BMI. Dotted lines, 95% confidence intervals.

multivariate models. Ninety-five per cent confidence intervals excluded large differences in % emphysema and alpha across quintiles of plasma carbonyl content.

Discussion

In this relatively large study of current and former smokers, we found plasma carbonyl content to be independently associated with neither lung function nor lung density. Although higher plasma carbonyl content was associated with lower FEV_1 in

Table III. Levels of carbonyls by severity of airflow obstruction in smokers.

	n	Carbonyls (nmol ml^{-1} plasma) Mean ± SD	Crude difference (95% CI)	Multivariate difference (95% CI)[b]	*p*-Value
Normal lung function	245	17.9 ± 2.9	0	0	Ref
Airflow obstruction (ratio <0.70)					
Mild (FEV_1 >80% pred)[a]	65	18.1 ± 2.6	0.35 (−0.4, 1.1)	0.40 (−0.4, 1.2)	0.33
Moderate (FEV_1 50–80% pred)[a]	138	17.8 ± 3.1	0.03 (−0.6, 0.6)	0.06 (−0.6, 0.7)	0.85
Severe (FEV_1 <50% pred)[a]	42	17.9 ± 2.5	0.17 (−0.8, 1.1)	0.01 (−1, 1)	0.99

[a]Based on pre-bronchodilator spirometric measures.
[b]Differences adjusted for age, sex, height, race/ethnicity, educational attainment, smoking status, packyears, body mass index, and physician diagnosis of asthma before age 45 years.

Table IV. Lung density in current and former smokers, according to quintiles of plasma carbonyl content.

	Quintile of oxidized protein					
	Q.1	Q.2	Q.3	Q.4	Q.5	*p* for trend
CT % emphysema	28.7 ($n=102$)	28.6 ($n=99$)	25.1 ($n=102$)	25.9 ($n=99$)	27.6 ($n=104$)	0.40
Difference in %emphysema (95% CI)[a]	0 (ref)	−0.34 (−4.78,4.09)	−4.14 (−8.55, 0.27)	−3.14 (−7.58, 1.30)	−1.96 (−6.37, 2.46)	0.23
Difference in %emphysema (95% CI)[b]	0 (ref)	0.51 (−3.83, 4.85)	−2.93 (−7.24, 1.37)	−2.31 (−6.71, 2.09)	−1.03 (−5.40, 3.35)	0.42
CT alpha	1.24 ($n=102$)	1.17 ($n=96$)	1.22 ($n=101$)	1.22 ($n=97$)	1.22 ($n=100$)	0.80
Difference in alpha (95% CI)[a]	0 (ref)	−0.03 (−0.12, 0.06)	−0.01 (−0.11, 0.08)	−0.004 (−0.10, 0.09)	−0.02 (−0.12, 0.07)	0.76
Difference in alpha (95% CI)[b]	0 (ref)	−0.06 (−0.14, 0.03)	−0.04 (−0.13, 0.04)	−0.03 (−0.12, 0.06)	−0.06 (−0.15, 0.03)	0.29

[a]Differences adjusted for age and sex.
[b]Differences adjusted for age, sex, height, race/ethnicity, educational attainment, smoking status, pack-years, body mass index, and physician diagnosis of asthma before age 45 years.

unadjusted analyses, this relationship was almost entirely explained by age-related differences in protein carbonyl content and FEV_1.

This is the first study of which we are aware to evaluate protein carbonyl content in COPD with appropriate control for smoking, although a number of prior studies have examined protein carbonyl content in relation to smoking. Increased oxidative stress in smokers has been shown by measuring various biomarkers, including lipid peroxidation products in plasma (Reilly et al. 1996, Bernert et al. 1997) oxidized DNA bases in leukocytic DNA and urine (Asami et al. 1996, Kiyosawa et al. 1999, van Zeeland et al. 1999) and F_2-isoprostanes in plasma and urine (Reilly et al. 1996, Morrow et al. 1995). Some of these biomarkers are also elevated in COPD (reviewed by Barnes 2004).

Nadeem et al. (2005) previously found increased plasma carbonyl content in COPD patients, most of whom were current or former smokers. Since the control group in that study was age-matched non-smokers, the increase in carbonyl content may have been due to smoking status rather than due to COPD. That study actually showed no association of plasma carbonyl content with lung function measures among the patients with COPD, which is consistent with our finding of no correlation between plasma carbonyl content and lung function among a much larger group of smokers.

Previous studies of protein carbonyl content have found that smokers had higher contents of protein carbonyls than non-smokers, using the conventional colorimetric assay (Lee et al. 1998), ELISA (Marangon et al. 1999a), and Western blot assay (Pignatelli et al. 2001). The Western blot study also examined differences in oxidized serum proteins between heavy and light smokers and found none. We also found no association of protein carbonyl content with cumulative or current smoking dose in the EMCAP study, although associations were found with age, ethnicity, educational attainment and obesity in this cohort (Yeh et al. 2008), in agreement with prior studies (Baynes 1991, Giugliano et al. 1996). Several studies have shown that protein carbonylation increases with the age of cells, organelles and tissues of varied species and has been associated to an exponential increase in molecular oxidative damage due to senescence of cells which leads to an imbalance with antioxidant mechanisms that occurs with age (reviewed by Chakravati & Chakravati 2007).

The present study has a number of strengths including relatively large size, lung density measures in addition to lung function, and confirmation of current smoking status by urinary cotinine. The ELISA method described in this study is sensitive and specific for oxidized proteins and, in our laboratory, has good reproducibility and has yielded results consistent with the prior literature (Marangon et al. 1999a). Power was adequate to detect clinically significant differences in lung function and density (for example, the 95% CI for FEV_1 excluded differences between the lowest and highest quintiles of protein carbonyls of larger than 140–170 ml). Protein carbonyls were measured on specimens stored for several years at -70°C. Stadtman and Levine (2003) have demonstrated the stability of protein carbonyls in storage for 10 years at -80°C and we have previously demonstrated expected associations in this cohort (Yeh et al. 2008).

There are a number of potential limitations of the present study. The cross-sectional design meant that participants with low lung function/density and COPD might have been more likely to take antioxidant supplements than healthy participants. If the supplements lowered their plasma protein carbonyls, this might have yielded a

negative result. Information on antioxidant supplementation use was available for a subset ($n = 134$) of this cohort. Controlling for antioxidant supplementation use in this subset did not alter results (data not shown), making the negative finding unlikely to be due to this bias.

Use of pre-bronchodilator rather than post-bronchodilator spirometry measures may have biased our results for protein carbonyls; however, this bias was unlikely to be severe given the cohort's smoking history. Post-bronchodilator spirometric data were available for a subset ($n = 89$) of the cohort and results for post-bronchodilator measures in that subset were qualitatively similar (data not shown). Furthermore, this limitation did not apply to lung density measures.

Few participants in this cohort had very severe airflow obstruction consistent with severe COPD; therefore, we cannot exclude that plasma protein carbonyls are elevated in very severe airflow obstruction. The available results, however, suggest no consistent increase in patients with severe COPD or in the few participants with very severe COPD.

Finally, the total antioxidant status of the subjects enrolled in this study was not assessed. Many studies have shown an inverse association between certain antioxidants such as vitamin C with chronic respiratory symptoms and with lung function (Schwartz & Weiss 1990, Hu & Cassano 2000). Others have observed that from a variety of antioxidants supplemented to smokers, the protein damage marker carbonyl content and the DNA-damage marker 8-OHdG were decreased after vitamin E supplementation (Lee et al. 1998) or inhibited by glutathione (Reznick et al. 1992) but not by ascorbic acid or metal ion chelating agents. Marangon et al. (1999b) similarly found no effect of vitamin C and α-tocopherol on the amount of protein carbonyls induced *in vitro* by 2,2′-azobis (2-amidino-propane) hydrochloride but did find and increase in carbonyl content after lipoic acid supplementation.

In conclusion, plasma protein carbonyls were associated with neither lung function nor density in this older group of current and former smokers after appropriate control for age and smoking. These results suggest that plasma protein carbonyls is a poor biomarker of the oxidative stress levels in COPD.

Acknowledgements

This study was supported by grants HL075476, HL077612, RR024156 and ES09089 from the National Institutes of Health.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

American Thoracic Society. 1995. Standadization of spirometry.1994 update. American Journal of Respiratory Critical Care 152:1107–1136.

Asami S, Hirano T, Yamaguchi R, Tomioka Y, Itoh H, Kasai H. 1996. Increase of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in human leukocytes by cigarette smoking. Cancer Research 56:2546–9.

Barnes PJ. 2004. Mediators of chronic obstructive pulmonary disease. Pharmacological Reviews 56: 515–548.

Barr RG, Stemple KJ, Mesia-Vela S, Basner RC, Derk S, Henneberger P, Milton DK, Taveras B. 2008. Reproducibility and validity of a handheld spirometer. Respiratory Care. In press.

Baynes JW. 1991. Role of oxidative stress in development of complications in diabetes. Diabetes 40(4): 405–412.

Berlett BS, Stadtman ER. 1997. Protein oxidation in aging, disease, and oxidative stress. Journal of Biology Chemistry 272(33):20313–20316.

Bernert JT Jr, Turner WE, Pirkle JL, Sosnoff CS, Akins JR, Waldrep MK, Ann Q, Covey TR, Whitfield WE, Gunter EW, Miller BB, Patterson DG, Jr., Needham LL, Hannon WH, Sampson EJ. 1997. Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. Clinical Chemistry 43(12):2281–2291.

Buss H, Chan TP, Sluis KB, Domigan NM, Winterbourn CC. 1997. Protein carbonyl measurement by a sensitive ELISA method. Free Radical Biological Medicine 23(3):361–366.

Celli BR, MacNee W. 2004. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. European Respiratory Journal 23(6):932–46.

Church DF, Pryor WA. 1985. Free-radical chemistry of cigarette smoke and its toxicological implications. Environmental Health Perspectives 64:111–126.

Chakravati B, Chakravati DN. 2007. Oxidative modification of proteins: age-related changes. Gerontology 53:128–139.

Giugliano D, Ceriello A, Paolisso G. 1996. Oxidative stress and diabetic vascular complications. Diabetes Care 19(3):257–267.

Halliwell B. 1996. Free radicals, proteins and DNA: oxidative damage versus redox regulation. Biochemical Society Transactions 24(4):1023–1027.

Henschke CI, Yankelevitz DF, Libby DM, Pasmantier MW, Smith JP, Miettinen OS. 2006. Survival of patients with stage I lung cancer detected on CT screening. New England Journal of Medicine 355(17):1763–1771.

Hoffman EA, Simon BA, McLennan G. 2006. State of the Art. A structural and functional assessment of the lung via multidetector-row computed tomography: phenotyping chronic obstructive pulmonary disease. Proceedings of American Thoracic Society 3(6):519–532.

Hu G, Cassano PA. 2000. Antioxidant nutrients and pulmonary function: the Third National Health and Nutrition Examination Survey (NHANES III). American Journal of Epidemiology 151(10):975–981.

Kiyosawa H, Suko M, Okudaira H, Murata K, Miyamoto T, Chung MH, Kasai H, Nishimura S. 1999. Cigarette smoking induces formation of 8-hydroxydeoxyguanosine, one of the oxidative DNA damages in human peripheral leukocytes. Free Radical Research Communications 11:23–7.

Lee BM, Lee SK, Kim HS. 1998. 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 Letters 132(1–2):219–227.

Marangon K, Devaraj S, Jialal I. 1999a. Measurement of protein carbonyls in plasma of smokers and in oxidized LDL by an ELISA. Clinical Chemistry 45(4):577–578.

Marangon K, Devaraj S, Tirosh O, Packer L, Jialal I. 1999b. Comparison of the effect of alpha-lipoic acid and alpha-tocopherol supplementation on measures of oxidative stress. Free Radical Biomedical Medicine 27(9–10):1114–1121.

Mishima M, Hirai T, Itoh H, Nakano Y, Sakai H, Muro S, Nishimura K, Oku Y, Chin K, Ohi M, Nakamura T, Bates JH, Alencar AM, Suki B. 1999. Complexity of terminal airspace geometry assessed by lung computed tomography in normal subjects and patients with chronic obstructive pulmonary disease. Proceedings of National Academy of Sciences USA 96(16):8829–8834.

Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ 2nd. 1995. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. New England Journal of Medicine 332(18):1198–1203.

Muller NL, Staples CA, Miller RR, Abboud RT. 1988. "Density mask". An objective method to quantitate emphysema using computed tomography. Chest 94(4):782–787.

Nadeem A, Raj HG, Chhabra SK. 2005. Increased oxidative stress and altered levels of antioxidants in chronic obstructive pulmonary disease. Inflammation 29(1):23–32.

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. 2001. Nitrated and oxidized plasma proteins in smokers and lung cancer patients. Cancer Research 61(2):778–784.

Pryor WA, Stone K. Oxidants in cigarette smoke. 1993. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. Annals of NY Academy of Sciences 686:12–27.

Rahman I, MacNee W. 1999. Lung glutathione and oxidative stress: implications in cigarette smoke-induced airway disease. American Journal of Physiology 277(6 Pt 1):L1067–1088.

Rahman I, Morrison D, Donaldson K, MacNee W. 1996. Systemic oxidative stress in asthma, COPD, and smokers. American Journal of Respiratory Critical Care 154(4 Pt 1):1055–1060.

Reilly M, Delanty N, Lawson JA, FitzGerald GA. 1996. Modulation of oxidant stress in vivo in chronic cigarette smokers. Circulation 94(1):19–25.

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

Schwartz J, Weiss ST. 1990. Dietary factors and their relation to respiratory symptoms. The Second National Health and Nutrition Examination Survey. American Journal of Epidemiology 132(1):67–76.

Snider GL. 1985. Distinguishing among asthma, chronic bronchitis, and emphysema. Chest 87(1 Suppl.):35S–39S.

Stadtman ER, Berlett BS. 1997. Reactive oxygen-mediated protein oxidation in aging and disease. Chemical Research Toxicology 10(5):485–494.

Stadtman ER, Levine RL. 2003. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 25(3–4):207–218.

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

Yeh CC, Barr RG, Powell CA, Mesia-Vela S, Wang Y, Hamade NK, Austin JH, Santella RM. 2008. No effect of cigarette smoking dose on oxidized plasma proteins. Environ Research 106(2):219–225.

Zielinska-Danch W, Wardas W, Sobczak A, Szoltysek-Boldys I. 2007. Estimation of urinary cotinine cut off points distinguishing non-smokers, passive and active smokers. Biomarkers 12(5):484–496.