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Cys34 Adductomes Differ between Patients with Chronic Lung or Heart Disease and Healthy Controls in Central London

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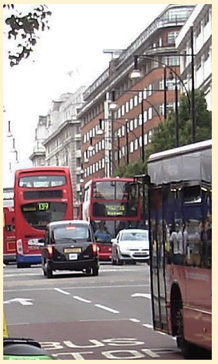
Supporting Information

ABSTRACT: Oxidative stress generates reactive species that modify proteins, deplete antioxidant defenses, and contribute to chronic obstructive pulmonary disease (COPD) and ischemic heart disease (IHD). To determine whether protein modifications differ between COPD or IHD patients and healthy subjects, we performed untargeted analysis of adducts at the Cys34 locus of human serum albumin (HSA). Biospecimens were obtained from nonsmoking participants from London, U.K., including healthy subjects (n = 20) and patients with COPD (n = 20) or IHD (n = 10). Serum samples were digested with trypsin and analyzed by liquid chromatography-high resolution mass spectrometry. Effects of air pollution on adduct levels were also investigated based on estimated residential exposures to $PM_{2.5}$, O_3 and NO_2. For the 39 adducts with sufficient data, levels were essentially identical in blood samples collected from the same subjects on two consecutive days, consistent with the 28 day residence time of HSA. Multivariate linear regression revealed 21 significant associations, mainly with the underlying diseases but also with air-pollution exposures (p-value < 0.05). Interestingly, most of the associations indicated that adduct levels decreased with the presence of disease or increased pollutant concentrations. Negative associations of COPD and IHD with the Cys34 disulfide of glutathione and two Cys34 sulfoxidations, were consistent with previous results from smoking and nonsmoking volunteers and nonsmoking women exposed to indoor combustion of coal and wood.



INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease, characterized by persistent air flow limitation, that affects about 170 million people worldwide[1] and is an increasingly important cause of disability and death.[2] Tobacco smoking is a well-established risk factor for COPD as are exposures to indoor air pollution from combustion of solid fuels, ambient air pollution, and some occupational chemicals.[3−6] The incidence of COPD has also been linked to increased risks of ischemic heart disease (IHD), which resulted in 15.9% of all worldwide deaths in 2015.[7] Risk factors for IHD also include smoking and air pollution, as well as hypertension, obesity, diabetes, lack of exercise, and psychosocial stress.[8,9] The causal links between both COPD and IHD and exposures to cigarette smoke and air pollution involve irritating gases and fine particles ($PM_{2.5}$) that trigger oxidative stress with the subsequent cascade of inflammation, carbonyl stress, mitochondrial injury and altered gene expression.[10,11] A common element of this progression is generation of reactive oxygen species (ROS) and other electrophiles that can modify functional macromolecules and reduce antioxidant defenses.

Despite their importance to disease processes, reactive electrophiles cannot generally be measured in blood because they have short life spans in vivo. For this reason, investigators have studied their dispositions by monitoring modifications to abundant proteins. Our laboratory has developed an untargeted "adductomics" method to investigate modifications at a highly nucleophilic cysteine residue (Cys34) of human serum albumin (HSA).[12] We focused on Cys34, not only because it efficiently scavenges small reactive electrophiles,[13,14] but also because its oxidation promotes a host of covalent modifications to circulating thiols[15] that can act as redox switches in homeostatic processes.[16,17] These Cys34 sulfoxidation products and disulfides represent potential biomarkers of oxidative stress and the redox state of the serum over the 1-month residence time of HSA (21 day half-life).[18,19]

We initially characterized adductomes with archived serum/plasma from healthy smokers and nonsmokers and discovered

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several adducts that were significantly associated with smoking.[12] Some associations had been expected, including higher levels of adducts of ethylene oxide, acrylonitrile and methylation in smokers. However, smoking produced *lower* levels of three Cys34 sulfoxidations that we had expected to be more abundant in smokers because of oxidative stress. In a more recent study, we performed Cys34 adductomics with serum from nonsmoking Chinese women, who were exposed to high levels of indoor pollutants from residential combustion of coal and wood.[20] Interestingly, Cys34 disulfides of the antioxidant glutathione (GSH) and two of its precursors, that is, γ-glutamyl cysteine (γ-GluCys) and cysteinyl glycine (CysGly), were present at *lower* concentrations in subjects exposed to combustion products than in controls. These preliminary studies suggest that the Cys34 adductome reflects a complex interplay of exposures and redox processes involving reactive oxygen species and antioxidants and that "normal" levels of some adducts can actually be reduced by exposures to inhaled pollutants.

McCreanor et al. reported that walking for 2 h along Oxford Street, a busy commercial road in central London, U.K., where traffic is restricted to diesel buses and taxis, was associated with significant reductions in lung function among asthmatics.[21] In a more recent study, participants with COPD or IHD were compared to healthy controls to evaluate the impact of walking along Oxford Street on respiratory and cardiovascular functions in elderly participants with chronic diseases (referred to as "the Oxford Street Study II").[22] Since oxidative stress contributes to COPD and IHD, we wished to determine whether Cys34 adductomics could differentiate global characteristics of the redox proteome (e.g., sulfoxidations and mixed disulfides)[17] between diseased and healthy subjects. Likewise, we were curious as to whether adductomes were influenced by urban air pollution. To test these hypotheses, we obtained serum samples from nonsmoking participants and matched controls in the Oxford Street Study II and also residential levels of air pollutants that had been estimated from spatial models for each subject.[22−25] After performing Cys34 adductomics with the serum, we compared adduct levels between healthy subjects and patients with COPD or IHD to find discriminating adducts and also investigated associations between adducts and levels of air pollutants.

MATERIALS AND METHODS

Serum Samples and Air-Pollutant Data. Archived serum samples, stored at −80 °C, were obtained from nonsmoking subjects (either never smokers or ex-smokers for at least 12 months) in the Oxford Street Study II, namely healthy participants (n = 20, 10 males and 10 females) and participants with either COPD (n = 20, 9 males and 11 females) or IHD (n = 10, all males). Subjects had been recruited with informed written consent under protocols approved by institutions who collaborated in Oxford Street Study II.[22] Demographic characteristics, including age, smoking histories, lung function and medications are given in Supporting Information (SI) Table S1. Two blood specimens were collected from each subject at the hospital laboratory: one at 8 a.m. prior to being exposed to air pollution for 2 h while walking on Oxford Street and another collected at 8 a.m. on the following day. Annual average air concentrations of ambient air pollutants ($PM_{2.5}$, NO_2, and O_3) at each residence were estimated from spatial models.[25] Pollutant concentrations varied between 0.27-fold and 1.12-fold across subjects ($PM_{2.5}$: 14.2−18.0 μg/m^3, median = 15.4 μg/m^3; NO_2: 23.6−50.1 μg/m^3, median = 36.2 μg/m^3; O_3: 37.5−51.7 μg/m^3, median = 43.5 μg/m^3).

Liquid Chromatography−Mass Spectrometry. Digested serum was analyzed by nanoliquid-chromatography high-resolution mass spectrometry (nLC-HRMS) to detect Cys34 adducts of HSA, as described previously in detail.[12] Briefly, samples containing approximately 0.1 mg of HSA (after methanol precipitation) were digested with trypsin using pressure cycling (Barocycler NEP2320, Pressure Biosciences Inc.) for 30 min at 37 °C. One microliter of each tryptic digest was analyzed with a LTQ Orbitrap XL HRMS coupled to a Dionex Ultimate 3000 nanoflow LC system using a nano-electrospray-ionization source operated in positive-ion mode (Thermo Scientific, Sunnyvale, CA). Peptides were separated at a flow rate of 750 nL/min with a Dionex PepSwift monolithic nanoflow column (100 μm i.d. × 25 cm). Mass spectra were acquired over the range m/z = 750 to 1000 using the Orbitrap mass analyzer. In data-dependent mode, up to six triply charged precursor ions were selected from each MS1 scan and fragmented by collision-induced dissociation and analyzed in the linear ion trap (MS2).

Sample Processing and Data Acquisition. Adducts were located in the third largest ('T3') peptide of HSA, with sequence ^{21}ALVLIAFAQYLQQC^{34}PFEDHVK^{41}. Duplicate specimens were processed in 10 batches. To adjust for variation in the amount of HSA digested in individual serum samples, peak areas of T3 adducts were normalized by the corresponding peak area of the adjacent HSA tryptic peptide that we refer to as a "housekeeping" (HK) peptide (^{42}LVNEVTEFAK^{51}). The peak area ratio (PAR, adduct peak area/HK peptide peak area) was used for statistical analyses. The rationale for using PARs for quantitation and their extensive validation with reference T3 adducts were described previously.[12] Values below the limit of quantitation (LOQ) were imputed a PAR of LOQ/$\sqrt{2}$ = 2.87 × 10^{-5} where the LOQ was estimated as the mean PAR observed for the seven smallest abundances of all putative T3 peptides. Added masses were calculated relative to the thiolate form of the T3 peptide (Cys34-S$^-$).

Statistical Analyses. Statistical analyses were performed for each putative T3 adduct as described previously,[12,20] using ln(PAR) as the dependent variable with SAS software for Windows (v. 9.4, SAS Institute, Cary, NC). Briefly, PARs were adjusted for batch effects, and subject-specific random effects were predicted from all replicate measurements using a linear mixed effects model (Proc MIXED of SAS). These predicted random effects were used for statistical analyses. First, Wilcoxon rank-sum exact tests (Proc NPAR1Way of SAS), were performed for T3 modifications with intraclass correlation coefficients (ICC) > 0.10 (n = 34), to determine whether adduct levels differed between COPD or IHD patients and healthy participants. Since males and females were available for COPD patients and healthy participants, the tests included both genders, whereas comparisons for IHD were performed for males only. Significance for multiple comparisons was gauged with a Bonferroni-corrected p-value = 0.00147. Finally, multivariable linear regression analyses (Proc REG of SAS) were performed, using the batch-adjusted ln(PAR) as the dependent variable and participant group, gender, age and residential levels of $PM_{2.5}$, NO_2, and O_3 as independent variables.

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Table 1. Putative T3 Peptides Detected in Serum from the Oxford Street Subjects

adduct	retention Time (min)	*m/z*, 3+, observed	Δmass (ppm)	PAR[d] (×1000) (median)	conc. (pmol/mg HSA)	mass (Da) added to T3 (Cys34-S⁻)	elemental composition mass added to Cys34-S⁻	putative annotation
OS1[a,b]	30.23	808.7320		0.066	0.196	−9.093		not Cys34 adduct
OS2[b]	30.51	810.4536		0.126	0.365	−3.928		not Cys34 adduct
OS3[a,b]	30.17	811.7620	3.27	1.821	5.252	−0.002	+H	T3 labile adduct
OS4[a,b]	30.61	811.7611	−2.17	9.940	28.726	0.000	+H	unmodified T3
OS5[a,b]	32.15	811.4257	0.002	3.068	9.143	2431.248	+ $C_{114}H_{172}N_{27}O_{30}S$	T3 dimer
OS6[a,b,e]	29.68	816.4197	−0.723	1.107	3.174	13.971	-H_2, + O	Cys34-Gln cross-link (monooxidation)
OS7[a,b]	31.00	816.4319	0.81	0.225	0.652	15.0262	+CH_3	methylation (not Cys34)[c]
OS8[b]	30.39	820.0921	−1.207	0.064	0.187	25.9952	+CN	*S*-addition of cyanide
OS9	30.10	821.0916	−0.743	0.059	0.171	28.9932	+ CHO	dehydrated form of OS12
OS10	30.29	821.7513	−0.706	0.020	0.058	30.9722	-H_2, + O_2	dehydrated form of OS13[c]
OS11[a,b,e]	29.68	822.4233	−0.851	1.415	3.976	32.9882	+HO_2	Cys34 sulfinic acid (dioxidation)
OS12	30.11	827.0946	0.09	0.053	0.149	47.0022	OS11+CH_2	Cys34 sulfinic acid plus methylation (not Cys34)
OS13[a,b,e]	29.93	827.7550	−0.870	0.200	0.595	48.9832	+HO_3	Cys34 sulfonic acid (trioxidation)
OS14[a]	29.63	841.0986	−0.725	0.129	0.375	89.0142	+$C_3H_5O_3$	*S*-addition of pyruvate or malonate semialdehyde
OS15[a,b]	30.41	841.7529	−1.224	0.496	1.422	90.9772	+$C_2H_3O_2S$	*S*-addition of mercaptoacetic acid
OS16[a,b,e]	29.59	845.4246	−0.804	0.727	2.126	101.9922	+C_3H_4NOS	*S*-Cys (−H_2O)
OS17[a,b]	31.58	847.1082	−1.818	0.059	0.175	107.0432	+C_7H_7O	*S*-addition of benzaldehyde or quinone methide
OS18[a,b,e]	28.71	851.4297	−2.666	220.280	645.764	120.0072	+$C_3H_6NO_2S$	*S*-Cys
OS19[a,b]	28.72	851.7571	−2.007	0.790	2.301	120.9972	+$C_3H_5O_3S$	*S*-Cys(NH_2 → OH)[c]
OS20[a,b,e]	29.19	856.1004	−1.262	14.856	43.119	134.0202	+$C_4H_8NO_2S$	*S*-hCys
OS21[a,b,e]	28.92	856.1011	−2.161	11.018	32.66	134.0222	+$C_4H_8NO_2S$	*S*-hCys
OS22	29.02	857.0876		0.123	0.354	136.9812		unknown
OS23[a,b]	28.71	858.7534	1.572	0.529	1.542	141.9792	OS_{18}+Na	Na adduct of *S*-Cys
OS24[a]	29.27	860.7717	−0.662	0.333	1.015	148.0342	OS21+CH_2	*S*-hCys, plus methylation (not Cys34)
OS25[a,b]	29.33	864.4318	−0.949	0.143	0.418	159.0142	+$C_5H_7N_2O_2S$	*S*-CysGly (−H_2O)
OS26[a,b]	29.71	865.4312	−0.277	0.049	0.141	162.0122	+$C_5H_8NO_3S$	*S*-(*N*-acetyl)Cys
OS27[a,b,e]	28.42	870.4369	−2.677	25.414	75.15	177.0292	+$C_5H_9N_2O_3S$	*S*-CysGly
OS28[a]	28.63	875.1062	0.251	0.896	2.663	191.0372	OS_{27}+CH_2	*S*-CysGly, plus methylation (not Cys34)
OS29[a]	27.29	894.1270		0.095	0.272	248.0992		unknown
OS30[a,b,e]	28.95	894.4429	−1.442	2.972	8.635	249.0472	+$C_8H_{13}N_2O_5S$	*S*-γ-GluCys
OS31	29.30	899.1140		0.078	0.222	263.0612		unknown
OS32[a,b,e]	28.80	913.4498	−1.171	2.551	7.109	306.0682	+$C_{10}H_{16}N_3O_6S$	*S*-GSH
OS33	29.18	918.1222		0.092	0.245	320.0852		unknown
OS34	27.11	927.1408		0.075	0.224	347.1412		unknown
OS35	32.21	928.7842		0.130	0.379	352.0712		unknown[c]
OS36[a]	27.68	931.8218		0.097	0.277	361.1842		unknown
OS37[a]	27.67	965.4928		5.581	15.639	462.1972		unknown
OS38	28.07	970.1643		0.260	0.756	476.2112		unknown[c]
OS39[a]	29.13	976.8207		0.060	0.175	496.1812		unknown

[a]Also observed by Lu et al.[20] [b]Also observed by Grigoryan et al.[12] [c]Adduct with intraclass correlation coefficient (ICC) < 10% [d]PAR, peak area ratio, adduct peak area/housekeeping peptide peak area. [e]Identity confirmed with reference standard.

RESULTS

Characterization of Adducts. More than 7000 peaks were located as possible T3 modifications in the 208 nLC-HRMS runs that were clustered into 83 adduct features. Manual curation eliminated 44 features that were not present in sufficient numbers of subjects for statistical analysis. This resulted in quantitation of 39 distinct T3-related adduct features (designated OS1 to OS39) as summarized in Table 1. As anticipated, adduct levels were essentially identical in blood samples collected from the same subjects on two consecutive days, consistent with the 28 day residence time of HSA. Over half of the features were annotated based on added masses, and MS2 spectra indicated that some were T3 modifications at sites other than Cys34. Most of these peptides had previously been observed in our laboratory,[12,20] including truncations (OS1 and OS2), a labile T3 adduct that disassociated in the electrospray (OS3),[20] unmodified T3 (OS4), T3 methylation (OS7), Cys34 oxidation products (OS6, OS11, and OS13), a likely product of reaction with either benzaldehyde or quinone methide (OS17) and 15 mixed

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Table 2. Mean Peak-Area Ratios (PAR) for Adducts with at Least One Significant Difference between Either COPD or IHD Patients and Healthy Subjects (Wilcoxon Test, p-Value ≤ 0.05)

		healthy subjects (n = 20)	COPD subjects (n = 20)		IHD Subjects (n = 10)	
adduct	putative annotation	PAR(×1000)	PAR(×1000)	p-value	PAR(×1000)	p-value
OS2	not Cys34 adduct	0.119	0.148	**0.01674**	0.120	0.91396
OS4	unmodified T3	9.470	10.620	**0.04595**	9.950	0.68112
OS6	Cys34-Gln cross-link (monooxidation)	1.209	1.044	**0.00427**	1.037	**0.01458**
OS11	Cys34 sulfinic acid (dioxidation)	1.536	1.302	**0.00353**	1.292	**0.01666**
OS12	Cys34 sulfinic acid plus methylation	0.058	0.050	**0.01674**	0.045	**0.00026**[a]
OS13	Cys34 Sulfonic acid	0.222	0.214	0.46117	0.184	**0.01897**
OS17	benzaldehyde or quinone methide	0.109	0.067	0.12735	0.047	**0.04783**
OS23	Na adduct of *S*-Cys	0.581	0.543	0.32726	0.465	**0.01897**
OS32	*S*-GSH	2.917	2.271	**0.00093**[a]	2.363	0.13073
OS33	unknown	0.104	0.079	**0.00159**	0.080	**0.04903**

[a]Significant association after Bonferroni adjustment (p-value < 0.00147).

Table 3. Results of Multivariate Linear Regression Models[a]

adduct	putative annotation	COPD	IHD	gender	age	NO_2	O_3	$PM_{2.5}$	adj. R^2
OS2	not Cys34 adduct	0.0323 (↑)							0.056
OS3	T3 labile adduct			0.022 (↓)					0.200
OS4	unmodified T3	0.0284 (↑)						0.0183 (↓)	0.166
OS6	Cys34-Gln cross-link (monooxidation)	0.0302 (↓)	0.0717 (↓)		0.0495 (↓)				0.313
OS11	Cys34-sulfinic acid (dioxidation)	0.0378 (↓)	0.0271 (↓)						0.292
OS12	Cys34 sulfinic acid plus methylation	0.0447 (↓)	0.0078 (↓)						0.238
OS13	Cys34 sulfonic acid					0.0477 (↓)	0.0426 (↓)		0.104
OS17	benzaldehyde or quinone methide	0.0247 (↓)				0.0174 (↑)			0.199
OS22	unknown	0.0317 (↑)		0.026 (↑)					0.187
OS32	*S*-GSH	0.0015 (↓)	0.0097 (↓)						0.193
OS33	unknown	0.0017 (↓)	0.0041 (↓)						0.199

[a]P-values and directions of associations are shown for the following covariates: COPD (COPD = 1; healthy subject = 0), IHD (IHD = 1; healthy subject = 0), gender (male = 1, female = 0). Results are only shown for models having at least one significant covariate effect (p-value < 0.05). Arrows indicate associations that either increased (↑) or decreased (↓) with the predictor variable.

Cys34 disulfides (the largest class of modifications). Nine adduct features, including dehydrated and methylated forms of Cys34 sulfoxidation products (OS9, OS10, and OS12) and six unknown adducts had not been detected in our previous investigations. The MS1 scans, selected ion chromatograms and MS2 spectra of the 9 unique adducts from this study are presented in SI Figures S1 and S2. Estimated PARs ranged from 2.0×10^{-5} to 2.2×10^{-1}, corresponding to approximate adduct concentrations of 0.058 to 646 pmol/mg HSA (Table 1). Annotations of 10 adducts (indicated in Table 1) were confirmed by comparisons with reference standards.

Associations of Adducts with Disease Status. Thirty four T3-related peptides, with ICC values greater than 0.10, were tested for differences between participant groups. As shown in Table 2, seven adducts potentially discriminated between COPD subjects and healthy participants (p-value < 0.05), notably two oxidation products (OS6 and OS11), *S*-GSH (OS32), a methylated oxidation product (OS12), and an unknown adduct (OS33). An additional subset of seven adducts potentially discriminated between the IHD subjects and healthy participants, including the Cys34 oxidation-plus-methylation product (OS12). Four of the associations were common to both COPD and IHD subjects (OS6, OS11, OS12, and OS33). Interestingly, all but two of the detected associations (OS2 and OS4 with COPD) reflected *lower* adduct levels in diseased subjects than healthy participants.

Multivariate Regression. Multivariate regression models were fitted for 34 adduct features, with results summarized in Table 3 for models having at least one covariate effect with a p-value < 0.05. All seven of the univariate associations for COPD (Table 2) were replicated after controlling for age, gender, and residential air-pollutant concentrations of $PM_{2.5}$, NO_2, and O_3 and two additional associations were detected (OS17 and OS22). However, two of the seven univariate associations with IHD (OS13 and OS17) did not replicate in the multivariate models and no additional associations with IHD were detected. Although air pollution levels were highly correlated across residences, with correlation coefficients all greater than >0.90 (not shown), they appeared to differentially affect adduct levels. For example, increased levels of NO_2 and O_3 were associated with decreased Cys34 sulfonic acid levels (OS13), whereas $PM_{2.5}$ appeared to have no effect on this adduct. On the other hand, increased $PM_{2.5}$ concentrations were associated with decreased levels of unmodified T3 (OS4), whereas exposures to NO_2 and O_3 had no apparent effect on this feature.

DISCUSSION

This is the first application of our adductomics methodology to investigate effects of respiratory and cardiovascular diseases and influences of ambient air pollution. We measured 39 Cys34 (and related) adducts in serum digests from 50 nonsmoking subjects in Oxford Street Study II, including 20 healthy participants, 20 with COPD and 10 with IHD. The Oxford Street Study II was designed to detect acute effects of urban air pollution (primarily diesel exhaust) during 2-h excursions of participants in a heavily polluted area of London, U.K.[22] Since

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Table 4. Comparison of Adduct Associations from the Current Study with Those of the Same Adducts in Two Previous Studies[a]

		current study[b]					smoky coal ref. [20,c]	smoking ref. [12,d]
adduct	annotation	COPD	IHD	NO_2	O_3	$PM_{2.5}$		
OS2	not Cys34 adduct	↑						↓
OS4	unmodified T3	↑				↓		
OS6	Cys34-Gln cross-link (monooxidation)	↓	↓					↓
OS11	Cys34 sulfinic acid (dioxidation)	↓	↓					↓
OS12	Cys34 sulfinic acid, plus methylation	↓	↓					
OS13	Cys34 sulfonic acid (trioxidation)			↓	↓			↓
OS17	benzaldehyde or quinone methide	↓		↑				
OS22	unknown	↑						↓
OS32	*S*-GSH	↓	↓				↓	
OS33	unknown	↓	↓					

[a]Associations with the indicated predictor variable at a p-value < 0.05 were obtained from multivariate linear regression models after controlling for covariates. Each arrow indicates the direction of the association with the predictor variable. [b]Covariates were age and gender. [c]Covariates were age and log-transformed levels of benzo(*a*)pyrene. [d]Covariates were race, gender, bmi, and consumption of animal fat and vegetable fat.

HSA turns over with a residence time of 28 days, we recognized that our application of adductomics was unlikely to detect effects of 2 h exposures and, indeed, adduct levels were essentially unchanged on consecutive days, before and after the excursions on Oxford Street. Rather, we focused on possible long-term effects of air pollutants as indicated by modeled exposures to $PM_{2.5}$, NO_2 and O_3 at each subject's residence.

Multivariate linear regression models detected 18 significant associations with underlying diseases or pollutant exposures, 14 of which were in the negative direction (adducts levels decreased with the presence of disease or higher air pollutant concentrations, Table 3). Five of the seven Cys34 adducts that discriminated for COPD (p-value < 0.05) were present at lower levels in COPD patients than in healthy subjects (Table 2), with ratios of COPD patients/healthy subjects ranging between 76% and 86% (median = 85%). The ratios of the seven discriminating adducts for IHD patients/healthy subjects ranged between 43% and 86% (median = 80%). Because our serum samples were from a cross-sectional study, it is not clear whether the detected associations between adduct levels and disease status were related to possible causal factors or to effects of the diseases themselves (reverse causality).

Four adducts were negatively associated with both COPD and IHD (three Cys34 oxidation products and the Cys34-GSH disulfide). This is interesting because we had found similar negative associations of the Cys34 oxidation products in smokers[12] and of the Cys34-GSH disulfide in women exposed to indoor combustion products[20] (Table 4). Since oxidative stress is a hallmark of COPD and cigarette smoking,[10] it is perhaps surprising that production of Cys34 oxidation products would be diminished in COPD patients and smokers. However, these results are consistent with dysregulation of pathways related to redox control of Cys34 due to hypoxia, which is characteristic of both COPD[26−28] and IHD[29−32] as well as chronic exposure to cigarette smoke.[33−36] Alternatively, differential rates of adduction could be related to structural and conformational specificities of reduced and oxidized forms of Cys34,[37,38] to interactions with other influential HSA loci (e.g., His39 and Tyr84),[39] and to nearby HSA binding of ligands such as fatty acids.[40,41] Reduced levels of Cys34-GSH in COPD and IHD patients are consistent with depletion of endogenous GSH by ROS as suggested by other avenues of research. For example, COPD patients had reduced concentrations of endogenous GSH in bronchoalveolar-lavage fluid[42] and lower levels of glutamylcysteine ligase, a key enzyme in glutathione synthesis, in alveolar macrophages and the bronchial epithelium.[43]

Although most of the detected associations were with disease status, suggesting that redox control was driven by the diseases, a few adducts were associated with residential concentrations of air pollutants that had been estimated from spatial models (Table 3). Inverse associations were observed between the Cys34 sulfonic acid (trioxidation product, OS13) and NO_2 and O_3 and between the unmodified T3 peptide (OS4) and $PM_{2.5}$. This behavior could again reflect dysregulation of redox control or could result from other exposure-related phenomena, such as altered Cys34 adduction due to binding at other sites on the HSA molecule or to altered rates of HSA turnover.[44] The weak positive association of NO_2 with the putative adduct of either benzaldehyde or quinone methide is perplexing because both benzaldehyde and quinone methide are primarily products of nutrients and microbial metabolites[45,46] that should not be sensitive to air pollution.

This was the first application of untargeted analyses to discover associations between adductomes and exposures to ambient air pollution. A few such associations were detected despite rather small exposure contrasts (pollutant concentrations varied between 0.27-fold and 1.12-fold across subjects). It would be interesting to further investigate adductomes related to wide concentration ranges of ambient air pollutants, such as when subjects are stratified between inner cities and rural locations. Regarding possible seasonal effects, blood samples from this study were evenly distributed over the time period from December 2012 to March 2014. Multivariate regression models indicated that season had no significant effect on adduct levels when other covariates (gender, age, and pollutant exposures) were included.

The above findings demonstrate that Cys34 adductomics offers a data-driven avenue for studies of respiratory and cardiovascular diseases and their connections with air pollutants. Despite small sample sizes, a variety of Cys34 modifications were associated with COPD, IHD and levels of air pollutants. These associations provide evidence that Cys34 adductomics can discover HSA modifications that discriminate across populations based on disease status and levels of pollutant exposures. By discovering unanticipated associations, Cys34 adductomics also highlights the value of untargeted analyses for generating hypotheses that can be pursued in subsequent investigations of the etiologies of respiratory and cardiovascular diseases. For example, serum metabolomics of

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reduced and oxidized forms of glutathione could be performed to test hypotheses related to GSH depletion and redox control.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b05554.

Additional information as noted in the text (PDF)

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S.L., S.R., S.D., and P.V. provided the conception and design; Acquisition, analysis, or interpretation of data were by H.G., W.E., R.S., P.C.(1), P.C.(2), K.F.C., B.B., and F.J.K.; Drafting or revision of manuscript was completed by S.L., S.R., H.G., R.S., P.C.(1), K.F.C., and PV.

Notes

The authors declare no competing financial interest.

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