aggressive, population-wide modalities of diagnostics, treatments, and secondary prevention (11). In support of this interpretation, a TB intervention in a community of 3,000 homeless people at Burnside, Oregon, that included skin testing, radiologic and sputum examination and treatment, coupled with education and outreach, obtained an 89% drop in active disease over the first 10 years of the program (12). ■

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Circulating Levels of Antioxidant Vitamins Correlate with Better Lung Function and Reduced Exposure to Ambient Pollution

To the Editor:

Particulate matter (PM) is possibly the ambient air pollution (AAP) that has the greatest effect on human health. Several studies consistently report an inverse association between PM exposure and lung function (e.g., FEV_1 and FVC) (1–6) and accelerated progression of chronic obstructive pulmonary disease (7) in adults. Furthermore, improved air quality associates with attenuated age-related decline in lung function (8).

Although several plausible mechanistic pathways have been described, the underlying mechanisms linking AAP and lung function have not been fully characterized.

High-throughput metabolomics approaches allow for an extensive set of small molecules to be measured in biological fluids. These metabolites represent pathways that reflect physiological functions, allowing for the potential identification of biomarkers (9).

To test the molecular links between lung function and AAP, we investigated the association between lung function and metabolomic parameters and between the same metabolites and exposure to PM (with aerodynamic diameter $\leqslant 10$ μm [PM_{10}] and $\leqslant 2.5$ μm [$PM_{2.5}$]) in the TwinsUK cohort.

Nontargeted metabolomic profiling using the Metabolon platform (280 metabolites) was performed in 5,519 fasting individuals from the TwinsUK cohort who also had undergone spirometry (Vitalograph model 2150, Buckingham, England), as described previously (10). A subset of 523 TwinsUK participants also had estimates of long-term exposure to PM at participants' postal code residences, derived from a 20×20 m dispersion model for London. All study participants completed a medical history and lifestyle questionnaire, including questions on vitamin supplementation (*see* online supplement for details) (11).

The study was approved by St. Thomas' Hospital Research Ethics Committee, and all participants provided informed written consent. TwinsUK data are publicly available on request on the department website (http://www.twinsuk.ac.uk/data-access/accessmanagement/).

We inverse-normalized the metabolomics data and excluded metabolic traits with more than 20% missing values. Metabolite associations with FEV_1 and FVC were assessed by random intercept

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This letter has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

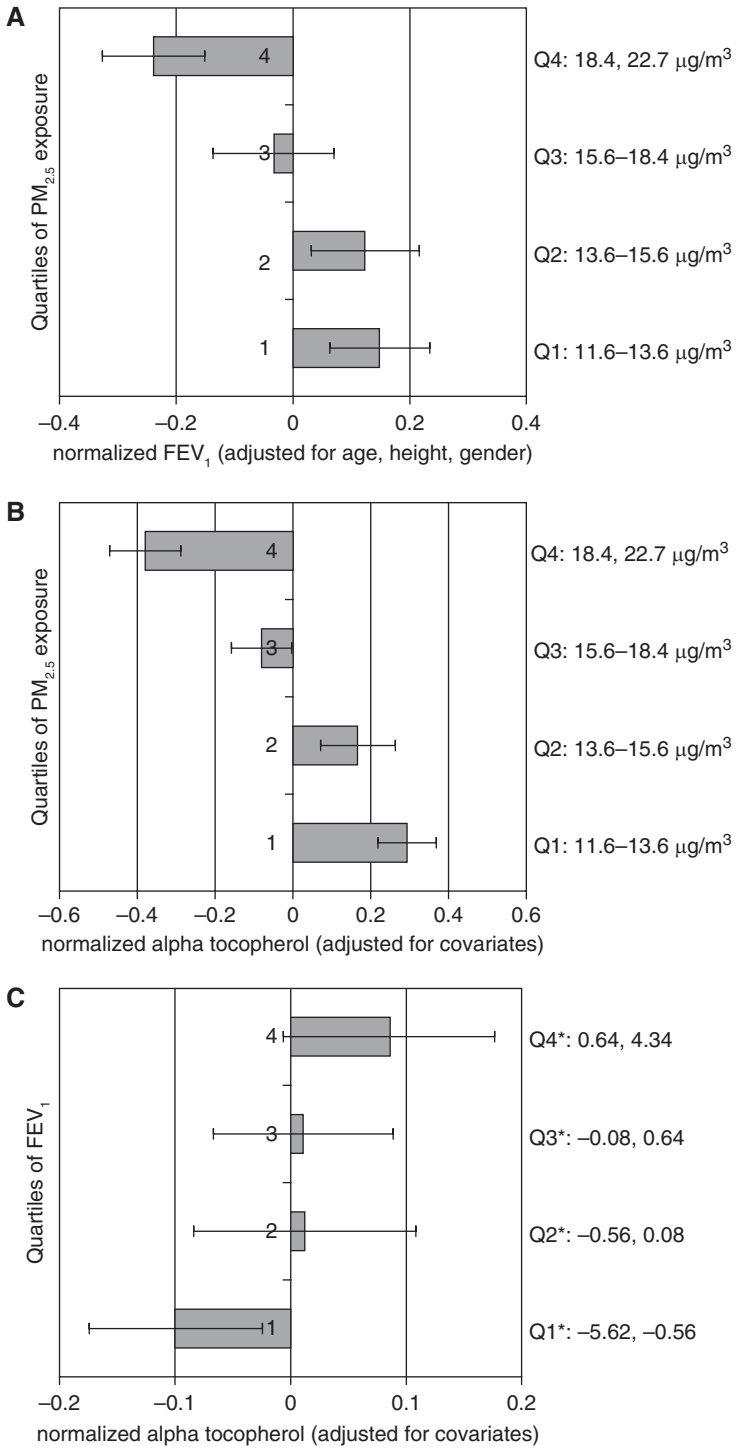

Figure 1. Associations among lung function, exposure to particulate matter with aerodynamic diameter ≤ 2.5 μm ($PM_{2.5}$), and α-tocopherol circulating levels. The minimum and maximum values for each quartile of $PM_{2.5}$ exposure (*A* and *B*) and FEV_1 (*C*) are shown. (*A*) Mean normalized FEV_1 adjusted for age, height, and sex among quartiles of $PM_{2.5}$ exposure. (*B*) Mean normalized α-tocopherol levels adjusted for age, sex, and batch among quartiles of $PM_{2.5}$ exposure. (*C*) Mean normalized α-tocopherol levels adjusted for age, sex, and batch among quartiles of FEV_1. *FEV_1 values were adjusted for age, height, sex, and body mass index and are normalized and standardized to have a mean = 0, SD = 1.

linear regressions adjusted for age, sex, body mass index, height, metabolite batch, and family relatedness. We adjusted for multiple testing, using Bonferroni correction, resulting in a significant threshold of 8.0×10^{-5} (=0.05/[280 metabolites × 2 traits]). The metabolites associated with FEV_1 and/or FVC were tested for correlation with $PM_{2.5}$ and PM_{10} after adjustment for covariates and multiple testing.

Table 1. Metabolomic Associations with Airborne Particulate Matter and Lung Function and Their Association with CRP

Metabolite	Super-p	Sub-p	$PM_{2.5}$		PM_{10}		FEV_1		FVC		CRP*	
			β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value
Asparagine	a-a	Alanine and aspartate metabolism	−0.1 (0.02)	5.68×10^{-8}	−0.05 (0.02)	9.09×10^{-4}	0.03 (0.01)	2.46×10^{-5}	0.02 (0.01)	6.09×10^{-3}	−0.05 (0.01)	1.10×10^{-5}
Glycine	a-a	Glycine, serine, and threonine metabolism	−0.11 (0.02)	9.06×10^{-9}	−0.07 (0.02)	3.52×10^{-5}	0.03 (0.01)	9.28×10^{-6}	0.03 (0.01)	2.03×10^{-6}	−0.08 (0.01)	1.20×10^{-12}
N-acetylglycine	a-a	Glycine, serine, and threonine metabolism	−0.12 (0.02)	9.11×10^{-10}	−0.08 (0.02)	4.63×10^{-7}	0.03 (0.01)	6.87×10^{-6}	0.04 (0.01)	3.93×10^{-9}	−0.04 (0.01)	8.20×10^{-5}
Serine	a-a	Glycine, serine, and threonine metabolism	−0.12 (0.02)	3.97×10^{-9}	−0.07 (0.02)	4.61×10^{-5}	0.03 (0.01)	3.07×10^{-5}	0.03 (0.01)	3.12×10^{-4}	−0.07 (0.01)	2.20×1
Glycerate	ch	Glycolysis, gluconeogenesis, pyruvate metabolism	−0.14 (0.02)	9.47×10^{-11}	−0.08 (0.02)	8.28×10^{-7}	0.03 (0.01)	2.06×10^{-7}	0.03 (0.01)	3.00×10^{-4}	−0.05 (0.01)	3.20×10^{-5}
Threonate	c&v	Ascorbate and aldarate metabolism	−0.12 (0.02)	8.96×10^{-9}	−0.08 (0.02)	1.99×10^{-5}	0.03 (0.01)	4.80×10^{-6}	0.02 (0.01)	1.29×10^{-3}	−0.04 (0.01)	5.30×10^{-5}
α-Tocopherol	c&v	Tocopherol metabolism	−0.17 (0.02)	4.92×10^{-13}	−0.12 (0.02)	3.48×10^{-10}	0.04 (0.01)	5.31×10^{-7}	0.04 (0.01)	3.57×10^{-6}	−0.03 (0.01)	6.30×10^{-3}
Benzoate	x	Benzoate metabolism	−0.11 (0.02)	6.02×10^{-6}	−0.07 (0.02)	3.13×10^{-4}	0.03 (0.01)	1.84×10^{-5}	0.03 (0.01)	1.67×10^{-5}	−0.01 (0.01)	1.80×10^{-1}

Definition of abbreviations: a-a = amino-acid; ch = carbohydrate; CRP = C-reactive protein; c&v = cofactor and vitamin; $PM_{2.5}$ = particulate matter with aerodynamic diameter ≤ 2.5 μm; PM_{10} = particulate matter with aerodynamic diameter ≤ 10 μm; x = xenobiotics.

*Log of CRP circulating levels. CRP was also tested for association with lung function and particulate matter. None of these comparisons reached nominal statistical significance ($P < 0.05$) after adjusting for the same covariates as for the metabolomics panel. The regression coefficients observed with logCRP were PM_{10} β (SE) = 0.009 (0.007), $P = 0.23$; $PM_{2.5}$ β (SE) = 0.013 (0.009), $P = 0.18$; FEV_1 β (SE) = −0.151 (0.253), $P = 0.565$; FVC β (SE) = −0.073 (0.360), $P = 0.843$.

The descriptive characteristics of the study population are shown in Table E1 in the online supplement. After adjustment for age, sex, body mass index, height, and family relatedness, exposure to $PM_{2.5}$ was seen to correlate negatively with both FEV_1 ($\beta = -0.03$; 95% confidence interval [CI], -0.04 to -0.01; $P = 0.001$; Figure 1) and FVC ($\beta = -0.02$; 95% CI, -0.04 to -0.01; $P = 0.01$). Similar results were found for PM_{10} (FEV_1: $\beta = -0.02$ [95% CI, -0.04 to -0.01; $P = 0.002$]; FVC: $\beta = -0.02$ [95% CI, -0.03 to -0.001; $P = 0.02$]).

FEV_1 correlates with 18 metabolites (Table E2), which fall into three principal classes: eight amino acids primarily involved in glycine, serine, and threonine metabolism; four cofactors and vitamins; and three lipids. There is also one carbohydrate, one nucleotide, and one xenobiotic.

The metabolomics analysis for FVC revealed 13 significantly associated metabolites, 10 of which were also identified for FEV_1 (Table E2). The associated metabolites were then tested for correlation with $PM_{2.5}$ and PM_{10} in a subset of 532 TwinsUK individuals living in the Greater London area.

Of the 21 metabolites associated with lung function, eight were also significantly associated with both $PM_{2.5}$ and PM_{10} (Table 1). Among the eight metabolites identified, four are amino acids, one is a carbohydrate (glycerate), one is a salt (benzoate), and two are cofactors and vitamins; namely, α-tocopherol and threonate. In all eight instances, a higher exposure to PM correlates with lower levels of the metabolite and a lower FEV_1 value (Table 1).

Seven of the eight metabolites identified correlate negatively with circulating levels of C-reactive protein, a marker of generalized inflammation (Table 1). The amino acids identified are all highly correlated with each other (Table E3), and in particular, glycine has been linked in the literature to pulmonary inflammation (*see* online supplement).

The strongest association both with $PM_{2.5}$ and FEV_1 was seen with vitamin E (Figures 1B and C). We also identified threonate, which is the major metabolite of ascorbic acid (vitamin C, a water soluble antioxidant) produced under oxidative conditions (12). We thus investigated use of vitamin supplements in study participants. We found positive significant correlations between use of vitamin supplements with both α-tocopherol ($\beta = 0.096$; SE = 0.05; $P = 0.045$) and threonate normalized levels ($\beta = 0.19$; SE = 0.06; $P = 0.001$). However, we found no correlation between use of vitamin supplements and $PM_{2.5}$ ($\beta = 0.02$; SE = 0.11; $P = 0.86$) and PM_{10} ($\beta = =0.03$; SE = 0.01; $P = 0.79$).

In conclusion, circulating levels of eight metabolites are significantly correlated with both exposure to AAP and lung function. The strongest association both with $PM_{2.5}$ and FEV_1 was with α-tocopherol levels: individuals with a higher $PM_{2.5}$ exposure have significantly lower levels of α-tocopherol and also have lower lung function. To our knowledge, this is the first report of significant association between α-tocopherol levels and $PM_{2.5}$ exposure in the general population. This is consistent with previous literature reports indicating that antioxidants, particularly α-tocopherol (but not γ-tocopherol), result in improved lung function (13), as well as with the extensive body of evidence indicating that lower α-tocopherol levels are observed in lung challenges such as asthma (*see* DISCUSSION in the online supplement).

α-Tocopherol is a biologically active form of the fat-soluble antioxidant vitamin E and also regulates gene expression (14). Supplementation with vitamin E reduces the damage to lung function caused by AAP in children (15). Circulating levels of α-tocopherol have also been shown to correlate positively with lung function in adults (13).

We note some study limitations: We could not access an independent population with PM and metabolomic data on which to confirm these results. Given the cross-sectional nature of the data, we were unable to make causal inference. However, in these data, use of vitamin supplements is positively correlated with circulating levels of both α-tocopherol and threonate, and as expected, there is no relationship between PM exposure and use of vitamins. Taken together, these findings suggest that subjects with lower levels of α-tocopherol are at greater risk of losing FEV_1 when exposed to urban PM. If this is indeed the case, the data may indicate that individuals with a high exposure to AAP would benefit from an optimal antioxidant status. ■

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Reduction of Airway Smooth Muscle Mass after Bronchial Thermoplasty: Are We There Yet?

To the Editor:

With interest, we read the letter by Pretolani and colleagues, who performed an observational study to investigate the effect of bronchial thermoplasty (BT) on airway smooth muscle (ASM) in patients with severe asthma (1). Preclinical studies have shown a reduction in ASM after BT that was associated with a reduction in airway hyperresponsiveness (2). However, the large randomized trials failed to reproduce this effect and showed only moderate clinical improvement in quality of life and exacerbation frequency (3, 4). The current study is the first that confirms reduction in ASM in patients with severe asthma after BT. The data show quite a dramatic (>45%) decrease in ASM in biopsies of BT-treated airways and, even more surprisingly, in biopsies of the non–BT-treated middle lobe. The proposed mechanism is that radiofrequent energy delivered during BT spreads its heat shock effect beyond the directly treated airway. This hypothesis is strengthened by the detection of ground-glass opacities around the non–BT-treated middle lobe in half of the patients. In our opinion, the findings described are very important; however, great caution should be made in drawing strong conclusions at this moment.

First, the high percentage of radiological abnormalities after BT observed by the French group has never been reported before, and is therefore unexpected. We can confirm this observation, as in our practice all patients after each BT procedure develop transient radiological abnormalities, mostly segmental atelectasis and/or peribronchial opacities. However, on a high-resolution computed tomography scan performed less than 24 hours after BT, no abnormalities could be detected in the non–BT-treated middle lobe.

Second, in the current study, ASM mass was analyzed in airway biopsies taken before and after BT at the same airway carinas, and the non–BT-treated middle lobe carina served as a control. Surprisingly, an unexpected decrease in ASM in the middle lobe after BT was observed. It cannot be excluded that (part of) the detected decrease in ASM is simply a scar effect of the prior biopsy. This effect especially applies for the middle lobe, as the anatomic area available for biopsies is very limited. In our opinion, this could be a plausible alternative explanation for the high-level decrease in ASM, as well as in the non–BT-treated middle lobe. Furthermore, as only a partial decrease in ASM after BT was seen in the earlier lobectomy study (5), it is hard to believe the effect of BT on ASM is this dramatic, even in distantly located non–BT-treated middle lobe airways.

In fact, ideally, BT-induced effects on the airway wall are assessed *in vivo* by a noninvasive technology that has high spatial resolution over a longer airway section. Optical coherence tomography (OCT), a light-based, near-histology, high-resolution imaging technology, is a very promising method to fulfill these requirements, as individual airway wall layers can be identified and measured longitudinally in an airway stretch in an accurate and reproducible way (6). Therefore, in the TASMA (Unravelling Targets of Therapy in Bronchial Thermoplasty in Severe Asthma) trial (ClinicalTrials.gov NCT 02225392), which is a randomized, international, multicenter trial to investigate BT targets, we use OCT, next to airway biopsies and standard X-ray-based imaging, to detect immediate and late effects of BT on airway wall layers, including ASM, and link these to clinical outcome. As such, we propose that OCT might qualify as an effect and/or screening technology for BT.

In line with this, we fully agree with our French colleagues that it is important and necessary to further unravel BT targets to ultimately improve patient selection for BT. ■

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