positive. We have started to assess the ability of DEP-pulsed bone marrow–derived dendritic cells to induce T_H17 differentiation of highly purified naive $CD4^+CD62L^+CD44^-$ cells and found that by itself DEP did not enable bone marrow–derived dendritic cells to promote T_H17 differentiation of naive $CD4^+$ T cells (data not shown). We are actively pursuing experiments to understand the mechanism by which *in vivo* DEP exposure results in T_H17 accumulation in the lung.

In our study, we confirmed that co-exposure of DEP and an allergen (in our case, house dust mite) markedly increased airway resistance, bronchoalveolar lavage fluid eosinophilia, T_H2 cytokines, and allergen sensitization.[4] Similar findings were made by numerous groups, including by Inoue et al, using different allergens, particles, and mouse strains, underscoring the important contribution of pollution-associated particles to experimental asthma exacerbation.[5] We have recently confirmed our findings in young 3- to 6-week-old BALB/c mice using 2 different doses of DEP.[6]

Estimates of DEP exposure were derived from a previously developed land-use regression model.[7] The modeled pollutant, elemental carbon attributable to traffic, was initially derived at ambient monitoring stations in the Cincinnati airshed using detailed receptor model analyses and is a surrogate marker of traffic-related air pollution primarily derived from diesel exhaust.[8] Thus, concentrations of elemental carbon attributable to traffic are not analogous to particulate matter less than 2.5 μm in diameter but, rather, represent the estimated DEP fraction of particulate matter less than 2.5 μm in diameter.

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Metabolomics of asthma

To the Editor:

Two recent publications in this *Journal* provide interesting reading regarding the metabolomics of asthma. Fitzpatrick et al[1] and Loureiro et al[2] identified metabolites and metabolic pathways suggesting that oxidative stress is a factor contributing to asthma severity in children and asthma exacerbation in adults, respectively. Although these findings support a role for oxidative stress in asthmatic patients, these studies have some limitations.

Because of the design of the studies, it is not clear whether the reported and highlighted metabolites are a proxy for oxidative stress contributing to severe asthma and asthma exacerbation or a consequence of the disease. It is also not completely clear whether the findings are driven by use of medication; this is common to both studies but particularly relevant in the study by Loureiro et al[2] because samples from the exacerbation period were collected after the treatment provided at the emergency department. The 2 groups of children from the study by Fitzpatrick et al[1] are not totally comparable because the group with severe asthma was almost exclusively nonwhite, unlike the control group (mild asthma), and because racial differences in biological predictors of severe asthma have been reported.[3] In addition, the use of nonfasting samples raises the question of whether the results might have been influenced by diet because several food items have been shown to associate with specific metabolites[4] and the short-term effects of diet on the metabolome are uncertain. Finally, like most of the previous studies on the metabolomics of asthma (Table I), these were pilot studies (small by nature) that could not adjust for potential confounders.

The studies by Fitzpatrick et al[1] and Loureiro et al,[2] together with previous studies, have shown several metabolites and pointed out several pathways that could characterize asthma or asthma exacerbations. Not all studies used the same methods (some used nuclear magnetic resonance and others used mass spectrometry) or sample types (some used serum and others used urine or exhaled breath condensate), collected fasting samples, or controlled for the use of asthma-related and non–asthma-related drugs or comorbidities. This should remind us of what we have seen when large genome-wide association studies did not replicate tens of single nucleotide polymorphisms that were previously found to be associated with disease in small candidate gene studies. Nevertheless, the present findings, as those from previous studies, are excellent candidate metabolites to be replicated in future and larger studies.

In summary, on the basis of the publications by Fitzpatrick et al[1] and Loureiro et al,[2] some might conclude that we have enough information to move to the development of treatments targeting the oxidative stress–related pathways. Unfortunately, we are still far from understanding the real meaning of these metabolites, and we cannot exclude that some of the current findings might be false-positive results that will not be replicated in larger and better controlled studies. Randomized trials and animal model studies showing the effects of controller drugs on the metabolic profile should provide important information that will help to disentangle the effect of such drugs on the metabolome.

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TABLE I. Summary of previous publications on the metabolomics of asthma and their main findings and issues

Reference (y)	Study population	Type of sample	Analytical method	Main findings	Main issues
Children					
Carraro et al (2007)[E1]	• 25 asthmatic patients (17 with persistent asthma treated with ICSs; 8 with intermittent asthma with no ICSs in previous month) • 11 healthy age-matched control subjects	Exhaled breath condensate	NMR	• Acetylated products (1.7-2.2 ppm spectral region) and oxidized compounds (3.2-3.4 ppm spectral region) discriminated asthmatic from nonasthmatic subjects	• Small sample size • Not clear what metabolites have been identified • Role of ICSs in findings not clear
Saude et al (2011)[E2]	• 73 with stable atopic asthma, 67% with ICSs • 20 with unstable asthma in emergency department, 50% with ICSs • 42 healthy age and sex matched (10 confirmed nonatopic)	Urine	NMR	• Several metabolites from several pathways discriminated asthma exacerbation from stable asthma	• Small sample size • Role of ICSs in findings not clear • Treatment given at emergency department not mentioned
Mattarucchi et al (2012)[E3]	• 41 atopic asthmatic patients (14 with SABAs; 16 with low-concentration ICSs + LABAs; 11 with high-concentration ICSs + LABAs) • 12 age-matched nonatopic nonasthmatic subjects	Urine	LC-MS	• Low levels of urocanic acid, methyl-imidazoleacetic acid, and a metabolite like isoleucyl-proline in asthmatic patients	• Small sample size • Role of ICSs in findings not clear
Sinha et al (2012)[E4]	• 58 asthmatic patients • 2 healthy nonasthmatic subjects	Exhaled breath condensate	NMR	• Asthmatic patients showed lack of ammonium ions	• Small sample size • No mention of use of ICSs or any other treatment
Carraro et al (2013)[E5]	• 42 atopic asthmatic patients (31 with non-severe asthma [17 with low- to medium-concentration ICSs alone or combined with LABAs mainly; 14 ICS naive); 11 with severe asthma with high-concentration ICSs (and others) + LABAs] • 15 healthy subjects	Exhaled breath condensate	NMR	• High levels of retinoic acid– and deoxyadenosine-related metabolites in patients with severe asthma • Low levels of ercalcitriol in patients with severe asthma • Low levels of 20-hydroxy-prostaglandin F_{2a}, 6-keto-prostaglandin F_{1a}, and thromboxane B_2 in patients with severe asthma	• Small sample size • Role of ICSs in findings not clear
Gahleitner et al (2013)[E6]	• 25 asthmatic patients (17 with persistent asthma treated with ICSs; 8 with intermittent asthma with no ICSs in previous month) • 11 healthy age-matched control subjects	Exhaled breath condensate (fasting)	GC-MS	• Eight metabolites discriminated asthmatic patients from healthy children: 1-(methylsulfanyl)propane, ethylbenzene, 1,4-dichlorobenzene, 4-isopropenyl-1-methylcyclohexene, 2-octenal, octadecyne, 1-isopropyl-3-methylbenzene, and 1,7-dimethylnaphtalene	• Small sample size • Role of ICSs in findings not clear
Adults					
Sinha et al (2012)[E4]	• 7 asthmatic patients (nonsmokers) • 10 healthy nonasthmatic subjects (nonsmokers)	Exhaled breath condensate	NMR	• Lack of ammonium ions in asthmatic patients	• Small sample size • No mention of use of ICSs or any other treatment
Ibrahim et al (2013)[E7]	• 79 asthmatic patients (77% with ICSs) • 34 control subjects	Exhaled breath condensate	NMR	• Five spectral regions distinguished asthmatic patients from control subjects (AUC = 0.91)	• Small sample size • Role of ICSs in findings not clear • Control subjects were approximately 14 y younger than asthmatic patients
Jung et al (2013)[E8]	• 39 asthmatic patients (77% with ICSs; they stopped their medication for ≥3 d before sample collection; 44% were atopic) • 26 healthy control subjects	Serum	NMR	• High levels of methionine, glutamine, and histidine and lower levels of formate, methanol, acetate, choline, O-phosphocholine, arginine, and glucose in asthmatic patients	• Small sample size • Medication stopped 3 d before sample collection, but ICS-related DNA methylation effect should not be excluded
Loureiro et al (2013)[E9]	• Woman with severe persistent allergic asthma treated with omalizumab	Urine	GC-MS	• Previous to treatment, main metabolites were alkanes; 12 weeks after, aldehydes were main metabolites	• Just 1 case
Ried et al (2013)[E10]	• 260 asthmatic patients from the KORA cohort (asthmatic patients with 3 definitions: 260 patients with asthma ever; 147 nonmedicated patients with current asthma; 104 medicated asthmatic patients) • 2778 patients with asthma never (control subjects)	Serum (fasting)	ESI-MS/MS	• High levels of phosphatidylcholines and low levels of lyso-phosphatidylcholines in patients with current asthma (similar results for medicated asthma) • Changes in levels of polyunsaturated phosphatidylcholines were associated with asthma and affected by asthma risk alleles (in *PSMD3* and *MED24*)	• Causality could not be established

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AUC, Area under the curve; *ESI-MS/MS*, electrospray ionization tandem mass spectrometry; *GC-MS*, gas chromatography mass spectrometry; *ICS*, inhaled corticosteroid; *KORA*, Kooperative Gesundheitsforschung in der Region Augsburg; *LABA*, long-acting β-agonist; *LC-MS*, liquid chromatography mass spectrometry; *NMR*, nuclear magnetic resonance; *SABA*, short-acting β-agonist.