Correspondence

Metabolomics in Exhaled Breath Condensates

To the Editor:

The intriguing article by Dr. Carraro and colleagues (1) concerning nuclear magnetic resonance (NMR) measurements of exhaled breath condensate (EBC) for distinguishing patients with asthma from normal subjects suggests that this procedure may be used as an "artificial nose" for detecting airway disease. Further studies of this novel approach should be encouraged, but two caveats are warranted.

In Carraro and colleagues' study, the EBC samples were not fully dried out, and they may have contained volatile substances derived from extrapulmonary sources. For example, more than 80% of exhaled NH_3 and vapors responsible for halitosis are derived from the upper aerodigestive structures (tongue, periodontal tissues, nose, sinuses, etc.) (2, 3), and contamination by volatile salivary compounds can have a profound effect on EBC pH (4). Volatile organic substances may reflect local bacterial metabolism and inflammation rather than abnormalities in the lungs. At the very least, NMR measurements should also be made in saliva. Additional studies are needed in subjects with tracheostomies or endotracheal tubes.

The EBC approach is particularly unsuitable for collecting volatile solutes since recovery of these substances in EBC is dependent on their distribution between the saliva, exhaled air and droplets, and the condensate, which can be altered by multiple factors including minute ventilation, salivary pH, solubility, temperature, and sample preparation.

Conflict of Interest Statement: R.M.E. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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From the Authors:

We appreciate Dr. Effros' comments on our article (1). The issue of oral contribution to exhaled breath condensate (EBC) is well accepted, although not fully clear (2). Lung auscultation can be contaminated by sounds coming from the upper airway tract. Spirometry can be affected by upper airway issues. So it is true for EBC. This fact does not lessen the value of any of these procedures. In addition, as reported in the article (1), our samples were almost completely dried. We decided not to dry them completely to avoid their precipitation and a consequent loss of the nonvolatile compounds. The drying process applied was nonetheless extensive (they were reduced to a volume of 10 μl), leading to the loss of most volatile compounds. Any residual volatile compounds were only at trace levels, and it is highly unlikely that they could have significantly affected our results.

We wish to stress that EBC samples were collected and processed in the same way for asthmatic and healthy children. The statistical methods used in the analysis of our results (pattern recognition methods) led to the identification of the compound patterns discriminating between healthy and asthmatic children, so we are confident that the differences identified are truly important for characterizing asthmatic subjects, whatever the exact source of each product within the respiratory tract. As Dr. Hunt says in his editorial (3), commenting on our article, these patterns may have a role both in classifying asthma subphenotypes and in identifying the biochemical disturbances that lead to asthma symptoms in each patient.

EBC is a new technique that still has its pitfalls, and we are well aware that our results need to be confirmed by further studies. The EBC technique nevertheless has great potential for the noninvasive study of the lung. Metabolomic analysis applied to EBC, enabling the simultaneous assessment of several biocompounds, definitively represents an innovative approach.

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Particulate Air Pollution in Irish Pubs Is Grossly Underestimated

To the Editor:

In their article, Dr. Goodman and colleagues evaluate the change in $PM_{2.5}$ levels in Dublin pubs after the Irish smoking ban (1). The $PM_{2.5}$ levels in pubs are most certainly log-normally distributed, so it is curious that the authors did not use a more appropriate statistical method to analyze these data, either by log-transforming the data or using nonparametric testing, as was done for the carbon monoxide and cotinine analyses. However, it is certain that the conclusion of a large improvement in indoor air quality after the smoking ban would be unaffected by this change.

More importantly, due to the limitations of the light scattering instrument used in this study, the Aerocet 531 Aerosol Particulate Profiler, the $PM_{2.5}$ concentrations presented in the paper are likely a gross underestimation of the true secondhand smoke (SHS)–derived particle levels. Any light scattering instrument does not measure mass concentration directly and therefore needs to be properly calibrated for the specific aerosol of interest, in this case SHS. With appropriate calibration of this device for measuring SHS, accurate SHS concentrations may be determined.

We performed this calibration for the Aerocet 531 by comparing this device to the standard gravimetric method using a Personal Environmental Monitor (PEM for $PM_{2.5}$). These experiments determined a calibration factor for the Aerocet 531 for measuring SHS of 8.3 (95% CI, 6.9 – 9.8). This means that the average preban $PM_{2.5}$ level in the Irish pubs was likely about 8.3 times higher than the level stated by Goodman and colleagues (1), or 295 $\mu g/m^3$. After adjusting the results of this study with the appropriate calibration factor, they are much more consistent with other similar studies (2–5).

The more accurate $PM_{2.5}$ concentration puts in perspective just how high the levels of particulate air pollution are in pubs with smoking. $PM_{2.5}$ concentrations of this magnitude are rarely if ever seen in outdoor air, are deemed "hazardous" by the U.S. Environmental Protection Agency, and are almost 30 times higher than the World Health Organization's target guideline for outdoor $PM_{2.5}$ concentrations. The article by Goodman and colleagues adds to the irrefutable body of evidence showing that smoke-free air policies improve air quality, save lives, improve health, and are cost-effective and popular.

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From the Authors:

In their letter, Drs. Travers and Lee correctly identify some differences between the levels of particles as observed in our study and those published in other studies. They state that, to measure secondhand tobacco smoke (SHS) specifically, a conversion factor needs to be applied to the Aerocet instrument. To our knowledge, no such conversion factor has been established and published in the peer-reviewed literature. Where authors have reported calibration of optical instruments to tobacco smoke, there seems to be no standardized way of doing this. Most reports measure smoldering cigarettes, not when a cigarette is actually smoked, nor the different types of cigarette. This clearly highlights the need for some level of uniformity in the approach to calibration to SHS that reflects the real situation encountered in pubs.

On the other hand, we did not set out to measure SHS. Our study was designed to measure $PM_{2.5}$, PM_{10}, and benzene levels in a range of public houses, pre– and post–smoking ban (1). We assumed that if there is a total ban on smoking in a venue, and it is obeyed, then the level of SHS should be zero. We wished to measure the effect of a smoking ban in pubs on the atmosphere, accepting that there are other sources of pollution in pubs. We wished to show that, when environmental tobacco smoke (ETS) is removed, whether it comes from SHS, side stream smoke, or smoldering cigarettes in ashtrays, the pollution load is significantly reduced. We were aware that the instrument as configured could not measure SHS exclusively, nor did we report our results as SHS. We claimed that the observed reduction in $PM_{2.5}$ was most likely due to a reduction in ETS. The PM instrument was used according to the manufacturer's instructions.

Our primary objective was to determine the change in levels, pre- and postban, where we observed an 83.6% reduction in $PM_{2.5}$, virtually identical to that reported by Travers and colleagues (2), and an 80% drop in benzene, both highly statistically significant, while at the same time we did not observe any significant change in indoor PM_{10}, or ambient outdoor $PM_{2.5}$, or PM_{10}.

Apart from the issue of a conversion factor to specifically convert our $PM_{2.5}$ values to SHS, there are other design differences that may influence the observed measurement levels. We measured levels in 42 pubs for a minimum of 3 hours in each venue on each occasion, both before and after the ban. Repace and colleagues (3) measured levels in seven bars, selected because of visible smoking, and measured for an average of 43 minutes per venue. Similarly, Travers and colleagues measured in 22 venues, 7 of which were bars and 6 bar/restaurants, again for a median time of 38 minutes. Edwards and colleagues (4) measured in 64 pubs in four groups, selected on socioeconomic grounds, monitoring for 30 minutes on average in the "busiest" room, where they showed the marked variation both between pubs and also within individual pubs, as we reported.

We also suggest that the composition of the cigarettes available in the different countries may be different, and produce variable amounts of combustion products. The surprising outcome from the various studies where there has been a comprehensive workplace smoking ban is that the relative reduction in measured particle levels seems remarkably consistent.

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