

# Correspondence

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## Useful Models of Asthma Need to Properly Phenotype Airway Narrowing

To the Editor:

In the March, 2011 issue of the *American Journal of Respiratory Cell and Molecular Biology*, Fedulov and Kobzik published a paper showing maternal transfer in allergically sensitized mice of CD11c<sup>+</sup> dendritic cells with altered DNA methylation (1). Although they argue that this process may be involved with the development of asthma in neonates, unfortunately the work is flawed by an overinterpretation of the functional measurement that relies solely on the empiric variable, “enhanced pause” or Penh, which has been shown to be primarily related to ventilatory timing and unrelated to airway resistance (2, 3). The consensus for the past 7 years among respiratory physicians and physiologists is that, by itself, the use of Penh is not to be trusted (4). This inability of Penh to adequately phenotype airway function was recently emphasized and confirmed in an extensive genetic association study with 29 mouse strains, which showed that Penh and airway resistance were clearly associated with different genomic regions (5).

The explanation given by the authors for using Penh is that it would be too difficult to measure pulmonary function in mice so young using an invasive method. This is an unacceptable justification for an inadequate measurement. Our concern echoes the unambiguous statement many years ago by the famous statistician John Tukey,

When the right thing can only be measured poorly, it tends to cause the wrong to be measured only because it can be measured well. And it is often much worse to have good measurements of the wrong thing (especially when, as so often is the case, the wrong thing will in fact be used as an indicator of the right thing) than to have poor measurements of the right thing. (6)

In their attempt to justify using the wrong thing, the authors cite references that “arguably” support their case. In fact these citations offer no such support. Without some mechanistic understanding and linkage, simply citing a correlation between Penh and direct measurements is no better than, say, citing a correlation between height and body weight in humans. In addition, several other references are simply included in support, even though they have nothing to do with the assumed correlation.

The seductive simplicity of the Penh measurement makes it attractive to investigators who lack the motivation and/or skill to make the proper measurement. With careful preparation, however, measurements of airway mechanical function can be made in small mice (7), and this should have been done, since without solid evidence of increased airway narrowing to Mch stimulation, the model has no relevance to asthma. The end result is that the authors spent considerable effort to show that DNA methylation of splenic dendritic cells can affect a mouse’s breathing pattern and perhaps sensitivity to inhaled irritants, a finding that might have some importance to the control of ventilation and its interaction with the adaptive immune system, but unfortunately provides little insight into the asthma pathology.

It is also of concern that this journal, which is to be commended for having the foresight to publish the consensus letter (4), seems to have overlooked the essential role that was ascribed to Penh in this recent paper (1). Insofar as the interpretation and problems with Penh have not changed, we urge the reviewers and

editors to ensure that the quantitative methodology reported in this journal adheres to high standards.

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## References

1. Fedulov AV, Kobzik L. Allergy risk is mediated by dendritic cells with congenital epigenetic changes. *Am J Respir Cell Mol Biol* 2011;44:285–292.
2. Lundblad LK, Irvin CG, Adler A, Bates JH. A reevaluation of the validity of unrestrained plethysmography in mice. *J Appl Physiol* 2002;93:1198–1207.
3. Mitzner W, Tankersley C, Lundblad LK, Adler A, Irvin CG, Bates JH. Interpreting Penh in mice. *J Appl Physiol* 2003;94:828–832.
4. Bates J, Irvin C, Brusasco V, Drazen J, Fredberg J, Loring S, Eidelman D, Ludwig M, Macklem P, Martin J, *et al.* The use and misuse of Penh in animal models of lung disease. *Am J Respir Cell Mol Biol* 2004;31:373–374.
5. Berndt A, Leme AS, Williams LK, Von Smith R, Savage HS, Stearns TM, Tsaih SW, Shapiro SD, Peters LL, Paigen B, *et al.* Comparison of unrestrained plethysmography and forced oscillation for identifying genetic variability of airway responsiveness in inbred mice. *Physiol Genomics* 2011;43:1–11.
6. Tukey JW. Methodology, and the statistician’s responsibility for both accuracy and relevance. *J Am Stat Assoc* 1979;74:786–793.
7. Bozanich EM, Janosi TZ, Collins RA, Thamrin C, Turner DJ, Hantos Z, Sly PD. Methacholine responsiveness in mice from 2 to 8 wk of age. *J Appl Physiol* 2007;103:542–546.

## The Penh Police Are Not Infallible

From the Authors:

Common practice in rebuttal letters calls for dry scholarship and genteel, if sometimes strained, etiquette. To make the discussion a bit more lively, we prefer to view ourselves as defendants in a science equivalent of an indictment, and we will follow what is by now a familiar legal strategy. First, we will vigorously dispute the accusations as if we were completely in the right. Next, following common practice to sway the court of public opinion, we will attempt to discredit our accusers. Finally, we will finish more gracefully with a proposed compromise that hopefully keeps us out of jail.

Our decision to use Penh measurements is based on three related arguments. First, the correlation between Penh and more invasive methods *for our experimental conditions* is sufficient to be useful (Reader: this sounds lukewarm, but

be patient). Several laboratories have found a robust correlation between Penh measurements and other assays of airway responsiveness in Balb/c mice, both at baseline and after allergen challenge in the ovalbumin model of allergic pulmonary inflammation (1–4), *the model and strain of mice we use*. This even includes data reported in the devastating analysis of the limitations of Penh in BL/6 mice by Dr. Irvin and coworkers (2). For example, they report a correlation of 0.809 ( $P < 0.05$ ) of Penh to airways resistance at baseline in “Table 1, where Penh is the ... parameter with the highest correlation to lung mechanics in the BALB/c mouse.” They also show correlation in allergen-challenged Balb/c mice in Figure 4 and state in the discussion: “We show that, although *Penh correlates with RL in the BALB/c mouse* [our italics], there is no significant correlation in the C57BL/6 strain.”

Second, we take the position that not all correlations are as trivial and without use, as implied by our accusers. Both clinical and experimental medical science make frequent and productive use of correlative observations. This is especially valuable when one type of observation or method is easier, cheaper, or more practical than another, and is useful even when the observation or method is arguably flawed, poorly understood, or not understood at all. Examples range from the correlative value of descriptive histopathology for prognosis (e.g., “small cell” carcinoma), to clinically useful but mechanistically mysterious physical diagnosis findings (asterixis, clubbing, café-au-lait spots, etc.) to laboratory tests (sedimentation rate, C-reactive protein, EEG tracings).

Third, performing invasive measurements of pulmonary function in very small mice is so challenging that one has to weigh the benefits versus the costs. Although we applaud the remarkable “motivation and/or skill” demonstrated in the paper reporting invasive analysis of lung function in 2-week-old mice (5), we expect most readers will agree that jugular venous cannulation, intubation, ventilation, and anesthesia in 7-g mice can be expected to be a difficult procedure to master. We note that none of the three articles reported in PubMed as citing this 2007 paper actually use the method to study young, small mice. We further note the absence in the literature of any other reports of successful invasive pulmonary function testing in mice this size. Also, we have reported previously that mice in our model allowed to age and reach a size where invasive measurements become easier, show similar changes to those seen using Penh (6). More recently, another group using our protocol also reported matching findings using both Penh measurements and Flexivent measurements (the latter were also performed on older mice) (7). It might take several months of a talented researcher’s time to master and conduct invasive studies of lung function on 2-week-old mice. If an existing, correlative assay can provide similar information, this would be an unproductive and profligate waste of research funds and arguably an unethical, unnecessary use of animals in terminal experiments. In contrast to our accusers, we consider this measurement to be a supportive part, not a central linchpin, of our experimental approach and findings reported in (8).

Hence, we consider the costs of acquiring the “motivation and/or skill” to perform invasive lung function assays in our model to be far greater than the benefits. We can summarize, and catch up to our accusers in the erudite quotation arms-race, by citing Voltaire: “le mieux est l’ennemi du bien,” or, as perhaps better paraphrased by President Clinton: “Don’t let the perfect be the enemy of the good.”

We now turn to the ad hominem attack section of our defense strategy. Unfortunately for us, there is a big problem. The truth is that our accusers are terrific, accomplished scientists who clearly have a commendable passion for rigor and high standards in science. The only snarky bone we can pick with them is that their considerable talents could arguably—and yes, we are fond of that word—be better applied to more productive and important activities.

The spirit of scientific exchange obligates us to consider seriously whether we might be wrong and whether we should alter our experimental design in the future to allow more study of older, larger animals with invasive methods to address the concerns of our accusers. We will do so. In the meantime, we propose an out-of-court settlement to our case. We completely deny all the charges, and state categorically and emphatically that we did absolutely nothing wrong, and we promise to (probably) never do it again.

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## References

1. Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand EW. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997;156:766–775.
2. Adler A, Cieslewicz G, Irvin CG. Unrestrained plethysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. *J Appl Physiol* 2004;97:286–292.
3. Finotto S, De Sanctis GT, Lehr HA, Herz U, Buerke M, Schipp M, Bartsch B, Atreya R, Schmitt E, Galle PR, et al. Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression. *J Exp Med* 2001;193:1247–1260.
4. Lee CC, Huang HY, Chiang BL. Lentiviral-mediated GATA-3 RNAi decreases allergic airway inflammation and hyperresponsiveness. *Mol Ther* 2008;16:60–65.
5. Bozanich EM, Janosi TZ, Collins RA, Thamrin C, Turner DJ, Hantos Z, Sly PD. Methacholine responsiveness in mice from 2 to 8 wk of age. *J Appl Physiol* 2007;103:542–546.
6. Fedulov A, Silverman E, Xiang Y, Leme A, Kobzik L. Immunostimulatory CPG oligonucleotides abrogate allergic susceptibility in a murine model of maternal asthma transmission. *J Immunol* 2005;175:4292–4300.
7. Midoro-Horiuti T, Tiwari R, Watson CS, Goldblum RM. Maternal bisphenol A exposure promotes the development of experimental asthma in mouse pups. *Environ Health Perspect* 2010;118:273–277.
8. Fedulov AV, Kobzik L. Allergy risk is mediated by dendritic cells with congenital epigenetic changes. *Am J Respir Cell Mol Biol* 2011;44:285–292.

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