The following is the abstract of the article discussed in the following letter:

**Adler, A., G. Cieslewicz, and C. G. Irvin.** Unrestrained plethysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. *J Appl Physiol* 97: 286–292, 2004. First published March 19, 2004; doi:10.1152/japplphysiol.01279.2004.—There has been significant utilization of the technique described by Hamelmann et al. (Am *J Respir Crit Care Med* 156: 766–775, 1997) in which a parameter, enhanced pause (Penh), related to airways responsiveness is noninvasively measured by unrestrained plethysmography (UP). Investigating this technique, we sought to answer these questions: 1) How do changes in Penh compare with changes in traditional plethysmographic and lung mechanical parameters? 2) How do UP parameters perform in two different mouse strains? Awake immunized and control BALB/c (n = 16) and C57BL/6 (n = 14) mice were placed in the UP chamber and exposed to doses of aerosolized methacholine while the following parameters were measured at each concentration: inspiratory time (TI), expiratory time (TE), total time (TTot), TI/Tot, peak inspiratory pressure, peak expiratory pressure, Pause, Penh, tidal volume (VT), VT/TI, VT/TE, and VT/Ti/Tot. The next day, lung resistance (RL) and compliance (CL) were invasively measured in the same animals. For the BALB/c, the parameters with the highest magnitude of correlation coefficient vs. RL are (in order) 1) CL, 2) Pause and Penh, 3) parameters of breathing frequency (TE, Ti/Ti, Ti), and 4) parameters related to VT (inspiratory pressure, expiratory pressure). Flow parameters (VTi/Tot, VT/TE, VT/Ti) and duty cycle parameters (Ti/Tot) had insignificant correlations. This ordering is significantly different in C57BL/6 mice, in which the parameters with the largest correlations are 1) CL, 2) parameters of breathing frequency, and 3) flow parameters. Pause, Penh, VT, and duty cycle parameters had insignificant correlations. These data show that Penh is problematic in the sense that it is strain specific; it behaves very differently in BALB/c and C57BL/6 mice. We suggest that UP parameters largely originate as part of reflex control of breathing processes, rather than in the lung mechanics and conclude that it is inappropriate to use UP parameters in general, and Penh specifically, as substitute variables for invasive mechanical indexes such as RL.

**Barometric whole body plethysmography in mice**

*To the Editor:* Assessing the value of unrestrained, barometric whole body plethysmography (bWBP) in different strains of mice, Adler et al. (1) recently reported on the correlation of bWBP parameters such as expiratory time (TE), Pause (= TE − TR/TE; TR is the time interval that encompasses 63% of the integrated expiratory pressure), and enhanced pause (Penh = PEEP/PIP × Pause; PEEP and PIP are peak inspiratory and expiratory pressure in the plethysmograph, respectively) with lung resistance (RL) in allergen-induced airway hyperresponsiveness to methacholine (MCh). As previously shown (3), this correlation was good in BALB/c mice, whereas in C57BL/6 mice it was absent. This study raises several concerns, and we disagree with its interpretations and generalizations.

1) The authors use a formula for Penh in which the ratio of PEEP/PIP is reversed. They identify the respiratory phase with elevated box pressure (Pb) as inspiration, (Fig. 1) and then seem to define TR as an inspiratory parameter starting from end expiration. It is unclear how they are deriving expiratory parameters like Penh on this basis. If TR is measured starting from end expiration (i.e., during inspiration) as Adler et al. state in both the RESULTS and DISCUSSION sections, this would misrepresent values like Pause and Penh, which are based on Pb during expiration. During expiration, conditioning effects are significantly smaller than during inspiration. In addition, in the region of transition from inspiration to expiration, conditioning effects are further reduced (2). In this region, Pb is dependent on compression effects. Penh cannot be derived during inspiration.

2) Changes in RL to MCh provocation detected by Adler et al. (1) in sensitized and challenged BALB/c mice are surprisingly small. A dose of 12.5 mg/ml of MCh resulted in an increase in RL of ~310%, and the maximal change after 50 mg/ml MCh was ~450%. Considerably stronger effects of MCh provocation (12.5 mg/ml) have been observed previously by Takeda et al. (4) with an increase in RL of ~800% in BALB/c mice sensitized and challenged according to the same protocol used by Adler et al. (1) (RL at PBS baseline: 0.67 ± 0.02 cmH2O·s·ml⁻¹; RL at 12.5 mg/ml MCh 6.09 ± 0.32 cmH2O·s·ml⁻¹).

Furthermore, in C57BL/6 mice, the experimental model of allergic pulmonary inflammation used by Adler et al. (1) induced decreases in RL after provocation and increases in lung compliance (CL) at baseline compared with saline-treated controls. This response is unexpected and diametrically opposite to responses observed by Takeda et al. (4) and many others in the same strain of mice. In the study of Takeda et al., allergen sensitization and challenge did not result in significant differences in baseline CL or RL, and provocation with both MCh and serotonin resulted in small but significant increases in RL and in a reduction of CL (4). We believe that using experiments with such atypical outcomes does not provide a sound basis for an investigation of the value of different lung function measurements in allergen-induced airway responsiveness.

3) Adler et al. (1) illustrate changes of TR at increasing doses of MCh and show that TR values change at low doses of MCh, returning to baseline at higher doses with changes in opposite directions in BALB/c and C57BL/6 mice. In addition, they claim that changes in TR largely account for increases in Penh at low concentrations of MCh. This is not supported by the data presented. A significant difference in TR between sensitized and control BALB/c mice in the absence of MCh is not reflected in a difference in Penh, whereas Penh values differ significantly between these animals at 3.1 mg/ml MCh without significant differences in TR values. This indicates that data showing TR itself have little meaning because TR only gains significance in relation to TE as described by the parameter Pause. The TR data presented do not support the conclusion by Adler et al. that Penh has important experimental problems and that it cannot be considered a valid measurement of airway mechanics.

4) A reliable determination of respiratory timing and in particular of the transition from inspiration to expiration is essential for parameters derived by bWBP. Adler et al. (1) demonstrate how small changes in the definition of the “end-expiratory point” (which should be the end-inspiratory point) result in major changes in TR, TR, Pause, and Penh (1). Reliable algorithms and high sampling rates (usually of 500 Hz) are required to obtain consistent data. Surprisingly, after stressing the importance of identifying the transition between inspiration and expiration, Adler et al. only used a sampling rate of 100 Hz.

As previously shown, the study by Adler et al. (1) confirms that differences in the response of individual mouse strains to MCh provocation after allergic airway sensitization are re-
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flected not only in bWBP parameters but also in measurements of Rt. and Cl. Their data describing a lack of correlation between bWBP parameters and Rt. in C57BL/6 mice cannot be used to suggest that bWBP parameters do not reflect mechanical changes in the airways. We agree that bWBP parameters may also be influenced by additional factors such as changes in respiratory regulation or loss of elastic recoil of the lung parenchyma. All of these factors are likely to interact in models of allergic pulmonary inflammation, resulting in (or alleviating) respiratory distress. Thus bWBP parameters are not a substitute for established parameters of lung mechanics, but they are useful parameters of respiratory distress, which are probably also influenced directly by mechanical changes of the airways. Parameters like Penh may be more useful in models with predominant bronchial hyperresponsiveness due to peribronchial rather than parenchymal inflammation as has been postulated for the BALB/c mouse model of asthma (4).

REFERENCES


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REPLY

To the Editor: We would like to thank Drs. Shwarze, Hamelmann, and Gelfand for their interest in our paper (1). In this study, we measured a number of parameters, including Penh, that can be derived from the simple and noninvasive measurement of pressure fluctuations within an enclosed chamber. We then compared these noninvasive responses with more traditional and invasive measures of lung mechanics in two strains of mice (BALB/c and C57BL/6) with and without allergic airways inflammation elicited by antigen (ovalbumin) immunization and challenge. We found that measurement of breathing frequency was every bit as good as the more elaborate indexes such as Penh, but we found that noninvasive measurements of breathing pattern did not assess lung mechanical responses adequately, particularly in the C57BL/6 strain.

Schwarze et al. now raise some minor issues for which we provide the following responses and thoughts.

1) Schwarze et al. correctly point out two errors in our paper. The variables Prs and Prp should be interchanged in both Fig. 1 and Eq. 3. We have reanalyzed all the data, and the results were unchanged: that is, the error is typographical and not analytical. We apologize for this error, thank the authors for bringing it to our attention, and a corrigendum will be forthcoming.

2) We disagree with Schwarze et al. that the changes in responsiveness are small and hence do “not provide a sound basis.” We would point out first that a change of 450% in Rt. is hardly trivial. The baseline Rt. between groups was not significantly different, and the differences in baseline Cl. are likely due to difference in the size of the animals within the two groups; but in any event, it is well known that in allergic models baseline mechanical parameters have no bearing on responses to MCh or other bronchoactive agents. Second, as these authors are well aware, the changes observed from experiment to experiment can vary considerably, especially with the system used in this and several of the other studies performed at that period of time. We have reviewed eight recent papers by Schwarze et al. in which Penh was measured for BALB/c and C57BL/6 mice (4–11). In terms of percent increase in Penh above baseline for control animals, our results are well within the range of reported values; in fact, in most cases our results are close to the average. For example, at 25 ng/ml, we report a 203% increase in Penh in the BALB/c mice; the minimum increase at this dose is 95% (10) and the maximum 269% (9). In the C57BL/6 mice, we report a 36% increase at this dose, in comparison to values of 36 and 84% for interleukin (IL)-5+/− and IL-5−/− transgenic strains, respectively (10, Fig. 2A). If our study “does not provide a sound basis,” then presumably the same can be said about Schwarze et al.’s own work. Moreover, we would go on to point out that one measure of any technique’s usefulness is its ability to measure small changes, i.e., sensitivity. Lastly, several other groups have reported similar findings where Penh fails to track changes in invasive lung mechanics in the C57 mouse as well as other mouse strains or experimental conditions.

3) Even after several rereads, we are unclear as to the precise objection raised by Schwarze et al. concerning Tr. Our point was to illustrate how marked differences in an example component of the elaborate Penh parameter can account for experimental differences. When one thinks about it, how could a time variable relate to lung mechanics unless effort-independent airflow limitation occurs and a mechanical time constant results? This situation is unlikely in 1) the mouse, where the airways are huge relative to the parenchyma and 2) during very mild bronchoconstriction, where no changes in Rt. or Cl. have occurred (3.1 mg/ml). Their arguments, we think, show the futility of relating measurements of breathing frequency and tidal volume to well-validated and interpretable measurements of mechanical measures of airways responsiveness.

4) Here we agree with Schwarze et al. that “reliable algorithms and high sampling rates are required to obtain consistent data.” For the data presented we had used sampling rates of 100 Hz, because this sampling rate resolves the signals adequately for calculation of resistance and compliance. Even after contacting the company we are uncertain as to what rate the commercial devise uses. It was only afterward, when we analyzed the data,
that we discovered another pitfall of this technique, that due to rapidly descending nature of the end inspiratory flow rate, a higher sampling rate is probably required to fully resolve the response. In this regard, we find it odd, given all the laboratories that use this technique every day, that a careful analysis of its shortcomings and pitfalls is only now coming to light. In particular, we and others (1, 2) have shown serious shortcomings to the use of breathing pattern (frequency and tidal volume) as a surrogate for traditional measures of the mechanical properties of the lung.

Lastly, we are happy to note that Schwarze et al. agree with us that genetic strain is a significant factor in airways responsiveness. We also agree with Schwarze et al. that other factors, such as “respiratory regulation” (assuming here that they mean neural ventilatory control) or a loss of elastic recoil, do or likely influence the measurements of WBP. However, unlike Schwarze et al., we think it highly unlikely that WBP and Penh in particular will ever replace traditional invasive measures of lung impedance. Accordingly, we agree with Schwarze et al. that “bWBP parameters are not a substitute for established parameters of lung mechanics.” As such, we are joined by a considerable number of our colleagues who also draw into question and voice caution as to the use of this method (2). At this point the onus is now on the practitioners of Penh to provide convincing evidence that noninvasive bWBP measures anything more than what Bert (3) originally developed the technique for: noninvasive measurement of breathing frequency.

REFERENCES

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