Reply to Agrawal and Ram

Andrew M. Hoffman
Tufts University, North Grafton, Massachusetts

Reply: We appreciate the thoughtful comments from A. Agrawal and A. Ram in their adjoining letter concerning 1) typos in the text (which have now been addressed by issuance of the Journal of a corrigendum), and 2) various aspects of originality and accuracy to measure specific airway resistance (sRaw), as described in Lofgren et al. (10). First, we would like to reiterate our view that the work of the late K. P. Agrawal (2, 9) impacted substantially the techniques developed in our laboratory for study of mice and larger animal species (3, 4). Specifically, K. P. Agrawal opened the door to measurement of sRaw during tidal breathing in animals (2) and humans (9), by providing a computational approach to avoid heating and humidification errors. These advances were particularly germane to applications of whole body plethysmography originally described by Dubois et al. (7) to conscious animals. Our data in a variety of species support those principles. However, the physical properties of the device we created for mice were distinct from K. P. Agrawal’s method in guinea pigs and the subsequent adaptation in mice by Das et al. (5). Their restrained whole body plethysmograph (RWBP) employed a dual chamber within a pressure plethysmograph. All of these dual-chamber-in-box systems require the use of a neck collar to isolate the head from the thorax, a problem we sought to avoid by using a face mask-pneumotachograph within a box (i.e., RWBP), a subtle but important refinement discussed in our paper. What A. Agrawal and A. Ram assert to be a “straightforward adaptation” of RWBP by K. P. Agrawal’s from guinea pigs to mice has not produced straightforward data. The baseline values for sRaw (or 1/sGaw, where sGaw is specific airway conductance) using the adaptation from K. P. Agrawal by Das et al. (5) appear to be 1 log greater than predicted on the basis of invasive and RWBP measurements made by Lofgren et al. (10) (0.4 – 0.6 cm s⁻¹) with values for sRaw (1/sGaw) reported to be 4.0 – 5.0 cm s⁻¹ (11, 12), even higher than recalled in their letter (2.1 cm s⁻¹). In our view, the best explanation for these higher values is the neck collar used for Das’ adaptation of RWBP, the same problem that creates higher values and considerable differences between investigators using double-chamber plethysmography (DCP). For DCP, the literature states that, without careful attention while sizing the “neck hole”, there can be alterations of ventilation (compression) or leak between chambers (6, 8). Otherwise, we do not see why sRaw-RWBP, according to Das et al. (5), should differ from sRaw-RWBP, according to our study (10). Hence, the originality in our work was a simple refinement that has removed an important potential source of error and discomfort to the mice. Concerning the values for sRaw-RWBP according to Lofgren et al. (10), contrary to Agrawal and Ram’s assertion, we measured <1% leak around the nares (see METHODS), so errors in sRaw due to leak in our study were negligible. Furthermore, we did not utilize “post hoc reconstructions” of X-Y plots; thus phase lag in recording flow-box volume was also eliminated as a possible source of error (see METHODS). Importantly, the contention that sRaw by RWBP was artificially low in our study is contrary to the evidence presented (i.e., invasive sRaw-forced oscillation technique = 0.095 cm s⁻¹, which was 1/5th of sRaw-RWBP vs. 1/19th of sRaw-DCP in our study; 4/5th of Raw is due to upper airways, so sRaw-RWBP was within 5% predicted sRaw). Until the accuracy and reproducibility of sRaw using dual chamber systems are also rigorously evaluated in mice, the apparent inconsistencies at baseline and after methacholine (6) will continue to create problems with interpretation, or, at the least, ongoing technical challenges for users. Because the baseline measurements of sRaw are valuable for longitudinal evaluation, as recently documented by Agrawal et al. (1), and dictate the slope (anchor point) of the dose-response curve, this discussion is more than academic. Concerning the diversion of airflow through the mouth, this is an innovative way to avoid nasal contributions to sRaw. However, the contributions of oropharyngeal resistance and air volume to sRaw, and the source of erratic breathing they observed (1) will need to be better characterized. It is gratifying that A. Agrawal and A. Ram have initiated this dialogue. With the increasing use of mice to model aspects of human asthma (13), we need to continue working to refine the methods of conscious measurements until the benefits clearly outweigh the controversies.

REFERENCES
10. Lofgren JLS, Mazan MR, Ingenito EP, Lascola K, Seavey M, Walsh A, Hoffman AM. Restrained whole body plethysmography for measurement of...

