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Method of nebulizer calibration and methacholine airway responsiveness

To the Editor:

Direct bronchoprovocation challenges are commonly conducted by using one of 2 methods: the 2-minute tidal breathing method or the 5-breath dosimeter method. Both methods are described in the current American Thoracic Society guidelines.¹ We recently compared these 2 methods,² and in doing so, we realized that nebulizer calibration for the 5-breath dosimeter method could be conducted in several ways. Current recommendations are that dosimeter nebulizer systems be calibrated while in use by a subject to deliver 9 μ L per breath.¹ However, calibration guidelines are not well described, and 2 plausible methods meet American Thoracic Society guidelines. We have documented that measured output is greater with a standard (exhalation to the room) compared with a rebreathe (exhalation into the nebulizer) calibration method.³ We therefore investigated the effect of calibration method on methacholine PC₂₀ values.

Twelve subjects (7 women; age, 33 ± 3.1 [$\pm$ SEM] years; height, 67 ± 1.0 in) with mild asthma and mild-to-moderate airway hyperresponsiveness (tidal breathing methacholine PC₂₀, ≤ 8 mg/mL) were recruited to the study. Subjects had an FEV₁ of 70% of predicted value or greater (3.07 ± 0.18 L; $84\% \pm 3.1\%$ of predicted value). As-needed inhaled salbutamol ($n = 12$) was withheld for 6 or more hours, and inhaled corticosteroid ($n = 2$) or cromolyn ($n = 1$) was continued at a stable dose (≥ 4 weeks). There were no respiratory infections or, where applicable, allergen exposure for 4 or more weeks. Ethics approval was granted, and signed informed consent was obtained.

Four DeVilbiss 646 nebulizers (Sunrise Medical HHG Inc, Somerset, Pa) containing 3 mL of sterile isotonic saline were calibrated in triplicate by a single calibrator (research technician) to deliver $90 \mu\text{L} \pm 10\%$ after 10 breath actuations (ie, 9 μL per breath) for each of 2

different methods. The standard method involved inhalation from functional residual capacity to total lung capacity, a 5-second breath hold, and then removal of the mouthpiece from the mouth and exhalation to the room. The rebreathe method involved inhalation from functional residual capacity to total lung capacity, a 5-second breath hold, and exhalation back into the nebulizer through the mouthpiece. The stand-alone KoKo Dosimeter (Pulmonary Data Services, Inc, Louisville, Colo) actuation time for both methods was 0.6 seconds.

Two methacholine challenges were done in the same way as their respective calibrations and in random order within 7 days at the same time of day. The nebulizer output was measured for each subject by using the calibrator's settings before both challenges. If the output differed by greater than 10%, the nebulizer was recalibrated by the subject to administer 9 μL per breath. Baseline spirometry, including full forced vital capacity maneuvers (baseline only), were performed in triplicate before diluent inhalation. Doubling concentrations of methacholine were then administered, beginning with a predetermined concentration and continuing until the FEV₁ measured 30 and 90 seconds after inhalation had decreased by 17% or more compared with that seen after the diluent. The time between the start of inhalation of one concentration to the start of inhalation of the next concentration remained constant at 5-minute intervals. Methacholine PC₂₀ values were then algebraically calculated from the log concentration versus response curves.^{4,5}

Log-transformed methacholine PC₂₀ values were compared with the paired *t* test (STATISTIX 4.1 Analytical Software, Tallahassee, Fla).

Results of the calibrator's studies are as follows. The output of the 4 nebulizers determined by using the rebreathe method with the standard settings was lower by about 30% (6.5 vs 9.3 μL ; $n = 4$; $P = .0008$). With the nebulizer settings increased to obtain the desired output by the rebreathe method, the standard calibration measurement at these settings resulted in a 50% higher measured output (13.3 vs 8.8 μL ; $n = 4$; $P = .003$).

The methacholine PC₂₀ value was a half concentration lower (more responsive) for the rebreathe method versus the standard calibration method. The geometric mean PC₂₀ values were 1.5 mg/mL (95% CI, 1.0-4.9) and 2.3 mg/mL (95% CI, 0.70-3.3), respectively. Individual values are shown in Fig 1.

A secondary observation was that the measured output was closer to the calibrator's for the standard versus rebreathe method ($7.3 \pm 0.5 \mu\text{L}$ vs $3.4 \pm 1.1 \mu\text{L}$, respectively; $P = .001$).

These results lead to 3 important observations. First, the rebreathe method of calibration produces a lower measurement of output by about one third than does the standard method, confirming our previous study.³ Second, increasing the nebulizer settings to achieve the desired 9- μL measured output by means of the rebreathe calibration method results in about a one-half concentration reduction in PC₂₀ value. Third, the standard method of calibration

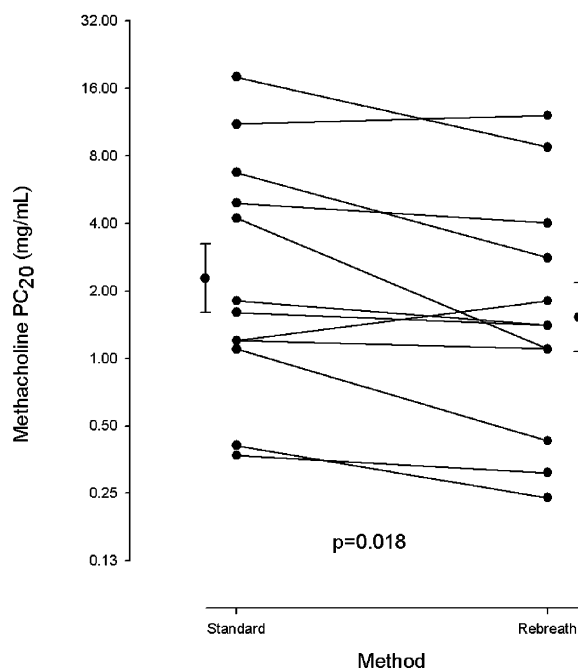


FIG 1. Methacholine PC₂₀ values (in milligrams per milliliter). The standard calibration method is on the left, and the rebreath method is on the right.

appears to be more repeatable between subjects than does the rebreath method.

Our previous investigation showed a similar one-third lower output with the rebreath method compared with the standard calibration by using 2 different dosimeters (KoKo and S&M).³ We hypothesized that the rebreath method underestimated the true output, likely because of condensation of exhaled water vapor in the nebulizer. The current investigation supports this view. The highly significant difference in PC₂₀ values with similar measured outputs (9 μ L per breath) by using the 2 methods confirms that the nebulizer outputs were not truly identical. In a small study this PC₂₀ difference was seen with the S&M dosimeter also,⁶ suggesting these findings are independent of dosimeter brand.

Although not one of our study goals, the observation that the rebreath output measurement is (much) more variable between subjects is not surprising. Individual calibration cannot be recommended as a routine procedure because in some subjects, an extra study day was required to complete the recalibration, a tedious and time-consuming task. The standard calibration depends mainly on the nebulizer settings and might be expected to be reasonably reproducible between individuals. By contrast, the rebreath output depends, at least in part, on subject characteristics that might influence the amount of water vapor exhaled. The most important of these would likely be the expired volume and possibly the expiratory flow rate. This aspect was not studied critically.

We did not investigate the KoKo DigiDoser Dosimeter system, and our findings might warrant critical examination of the output produced by this fully automated and

nonadjustable system. We understand that the only possible method of bronchoprovocation and calibration requires a rebreath procedure (L. Hendeles, personal communication). The intersubject variability that we observed with respect to measured output with the rebreath method raises concerns surrounding the output produced by this system.

A one-half concentration difference in PC₂₀ is within the range of repeatability and has been arbitrarily considered the minimum clinically significant difference for research investigations. A systematic underestimation of the PC₂₀ value, even by a half concentration, would lead to misclassification of methacholine tests.

We believe that the standard method of calibration more accurately reflects the nebulizer output. When added to its better between-subject repeatability, we recommend that dosimeter nebulizer systems should be calibrated by what we have arbitrarily called the standard method.

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Glucocorticoid receptor α : β ratio in blood mononuclear cells is reduced in cigarette smokers

To the Editor:

Glucocorticoids are the most effective therapy currently available for treatment of asthma and are recommended in international guidelines.¹ A subset of patients with asthma fail to demonstrate an adequate therapeutic response to glucocorticoids, and this phenotype is termed *glucocorticoid-resistant* or *insensitive* asthma.² Several mechanisms have been postulated to account for the reduced response