

FIG 2. TNF- α expression *in situ*. Cryostat sections of patient's lesional skin before (**A**) and 24 hours after (**B**) treatment with anti-TNF antibody (infliximab) and of a healthy control subject (**C**) were stained with monoclonal mouse anti-human TNF- α antibody. Arrows in Fig 2, A, indicate high TNF- α expression in the epidermis and dermis. **D**, TNF- α mRNA expression was quantified before and after TNF- α treatment and as a control in normal skin.

end, total RNA was extracted from skin biopsy specimens with Trizol reagent (Invitrogen, Carlsbad, Calif). *TNFA* mRNA was quantified by using a TNF- α TaqMan gene expression assay on a 7300 Real Time PCR System (Applied Biosystems, Foster City, Calif), according to the manufacturer's indications. This reduction of TNF- α expression on the mRNA and protein level was paralleled by a dramatic recovery from erythema and a complete stop of epidermal detachment. It is highly interesting that neutralization of the cytokine TNF also downregulates its mRNA production. TNF- α is first produced as a transmembrane protein with an intracellular tail. Membrane-bound TNF- α is secreted by cleavage of the extracellular part by metalloproteinases. Infliximab has been shown to bind to soluble and membrane-bound TNF- α and in addition to induce apoptosis of TNF- α -producing cells, especially of activated T cells.⁸

The rapid resolution of skin lesions in TEN after systemic anti-TNF- α therapy with infliximab opens new perspectives for the treatment of this rare but life-threatening condition and strongly supports the hypothesis that TNF- α produced by keratinocytes, as well as monocytes/macrophages, is significantly involved in the tissue damage.

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REFERENCES

- Viard I, Wehrli P, Bullani R, Schneider P, Holler N, Salomon D, et al. Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. *Science* 1998;282:490-3.

- Wolff K, Tappeiner G. Treatment of toxic epidermal necrolysis: the uncertainty persists but the fog is dispersing. *Arch Dermatol* 2003;139:85-6.
- Lebwohl M. Psoriasis. *Lancet* 2003;361:1197-204.
- Paquet P, Nikkels A, Arrese JE, Vanderkelen A, Pierard GE. Macrophages and tumor necrosis factor alpha in toxic epidermal necrolysis. *Arch Dermatol* 1994;130:605-8.
- Nassif A, Moslehi H, Le Gouvello S, Bagot M, Lyonnet L, Michel L, et al. Evaluation of the potential role of cytokines in toxic epidermal necrolysis. *J Invest Dermatol* 2004;123:850-5.
- Fischer M, Fiedler E, Marsch WC, Wohlrab J. Antitumor necrosis factor- α antibodies (infliximab) in the treatment of a patient with toxic epidermal necrolysis. *Br J Dermatol* 2002;146:707-9.
- Prins C, Vittorio C, Padilla RS, Hunziker T, Itin P, Forster J, et al. Effect of high-dose intravenous immunoglobulin therapy in Stevens-Johnson syndrome: a retrospective, multicenter study. *Dermatology* 2003;207:96-9.
- Van den Brande JM, Braat H, van den Brink GR, Versteeg HH, Bauer CA, Hoedemaeker I, et al. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. *Gastroenterology* 2003;124:1774-85.

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Nebulizer output for methacholine challenges with the KoKo Digidoser

To the Editor:

Unrecognized variation in nebulizer output can affect the results of bronchoprovocation tests¹ because the apparent provocation concentration of bronchoconstrictor causing a 20% decrease in FEV₁ (PC₂₀) might be different from the true value. Accordingly, the American Thoracic Society (ATS) guidelines for methacholine challenge tests recommends that the output of a nebulizer be determined before it is placed into service and periodically thereafter.² For the 5-breath dosimeter method, the guideline recommends that the nebulizer be calibrated to deliver 9 μ L per 0.6-second actuation (equivalent to 0.900 $\pm$ 0.09 mL/min). We attempted to follow this guideline for the KoKo Digidoser (model no. 323200; Ferraris Respiratory, Louisville, Colo) and found that the nebulizer output was low and could not be calibrated to meet this requirement.

The KoKo Digidoser is a commonly used dosimeter in the United States for methacholine challenges. It is an all-in-one system combining spirometer and dosimeter in one handheld unit connected by a cable to a computer containing the challenge software (Fig 1, upper panel).

It uses a DeVilbiss 646 nebulizer (Sunrise Medical, Somerset, Pa). Also, Ferraris Respiratory sells an optional “Characterized” DeVilbiss 646 nebulizer (model no. 492249). With this unit, the capillary tube and baffle are welded into place to reduce variability, and the output is determined *in vitro* at the factory. The output is inscribed on the nebulizer bowl, along with a serial number. This nebulizer contains a regulator valve that limits inspiratory flow to 0.5 L/sec to further reduce variability. The system is driven by compressed air at a constant pressure of 30 psi, and the manufacturer-determined output is entered into the software program that controls the challenge. The value entered does not affect the PC₂₀ calculation but does affect the reported cumulative dose producing a 20% decrease in FEV₁. In contrast, the KoKo dosimeter (model no. 414400, Ferraris Respiratory), also known as the Rosenthal Dosimeter, is a stand-alone dosimeter, using the same DeVilbiss nebulizer (Fig 1, lower panel). The tank flow and pressure can be adjusted to calibrate nebulizer output according to ATS recommendations. A separate spirometer and challenge software are required to perform challenges with this device.

We determined the outputs of 7 characterized nebulizers in quadruplicate with the Digidoser system. In brief, each nebulizer was weighed on an electronic balance accurate to 0.5 mg before and after a technician inhaled saline at room temperature 10 consecutive times. The software program was set to deliver the dose over the conventional 0.6-second period. The system required the technician to exhale into the nebulizer (rebreath method) and would not activate if the technician exhaled into room air (standard method). The weight loss was converted to output in milliliters per minute. In addition, the outputs of 2 of these nebulizers were determined in a similar manner by using the stand-alone Rosenthal Dosimeter set at 30 psi and a tank flow of 5 L/min. With this dosimeter, it is possible to compare outputs by using both the rebreath and standard methods.

The statistical difference in measured and manufacturer-labeled outputs was determined with ANOVA.³ Nebulizers were modeled as a random effect, and trials were modeled as repeated measures.

The mean outputs of the 7 nebulizers determined with the Digidoser ranged from 0.178 to 0.579 mL/min and were markedly lower than the manufacturer’s labeled output of 0.830 to 1.028 mL/min ($P < .0001$, Table I). In contrast, when determined with the Rosenthal dosimeter, the outputs of 2 of these units were similar to the manufacturer’s labeled outputs by using the rebreath method ($P = .3$) but much higher when exhaling into room air ($P < .0001$). There was no difference in outputs when a different operator performed the inhalations. Outputs of 2 of the same nebulizers were determined with a new Digidoser, and similar results were obtained (data not presented). Thus the lower output was not a result of a malfunctioning of the Digidoser unit used for all 7 nebulizers.

These results suggest that output of the characterized nebulizers is about 60% lower when using the Digidoser, and this might have clinical implications. If, in fact, less

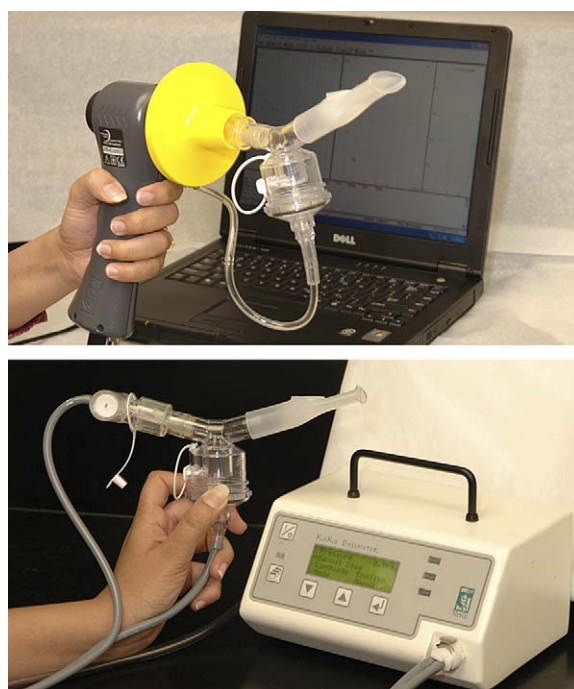


FIG 1. The KoKo Digidoser, an all-in-one handheld spirometer and dosimeter (upper panel): a characterized DeVilbiss 646 nebulizer is attached through a filter to the Digidoser, which, in turn, is connected by a USB cable to a computer. The KoKo dosimeter is a stand-alone dosimeter using the same DeVilbiss nebulizer that is connected directly to the dosimeter (lower panel).

methacholine is delivered during a challenge and the nebulizer cannot be calibrated, the apparent PC₂₀ is likely to be higher than the true value. Thus if a cutoff point of a PC₂₀ of 16 mg/mL or greater is used to exclude the diagnosis of current asthma, a patient with asthma might receive a misdiagnosis of being nonasthmatic (ie, a false-negative test result).

It is unclear whether the lower measured outputs with the Digidoser are an unexpected characteristic of that system or simply that the ATS-recommended method of determining output cannot be used with this dosimeter. A clinical study of methacholine PC₂₀ values using both dosimeters in the same subjects in a cross-over design is required to determine the clinical relevance of these findings.

It is noteworthy that a recent study indicates that the measured output of the standard DeVilbiss 646 nebulizer (ie, not characterized) with the Rosenthal dosimeter is one third less when the subject exhales into the nebulizer (rebreath method) compared with when the subject exhales into room air (standard method).⁴ A possible explanation for this difference is that exhaled breath contains water vapor that condenses in the nebulizer, thus reducing the difference in apparent weight loss during output determination. When the rebreath method was used to calibrate uncharacterized nebulizers to deliver 0.9 mL/min, as recommended in the ATS guidelines, the resultant methacholine PC₂₀ in asthmatic subjects was

TABLE I. The mean (range) of outputs from characterized De Vilbiss 646 nebulizers using the KoKo Digidoser and the Rosenthal Dosimeter

Nebulizer no.	Manufacturer-labeled output (mL/min)	Digidoser (mL/min)	Rosenthal Dosimeter rebreathe method* (mL/min)	Rosenthal Dosimeter standard method† (mL/min)
1250	0.883	0.327 (0.274-0.351)		
1251	0.886	0.473 (0.302-0.735)		
1538	0.965	0.579 (0.515-0.699)		
1541	0.830	0.178 (0.139-0.203)		
3634	0.901	0.256 (0.208-0.307)	0.912 (0.860-0.980)	1.50 (1.42-1.56)
3856	0.841	0.233 (0.186-0.275)	0.800 (0.520-0.960)	1.42 (1.36-1.54)
3900	1.028	0.491 (0.346-0.626)		
Mean (SD)	0.897 (0.06)	0.362 (0.15)‡	0.856 (0.07)	1.46 (0.05)§

*With the rebreathe method, the operator exhales into the nebulizer.

†With the standard method, the operator exhales into the room air.

‡Significantly lower than the manufacturer-labeled output ($P < .0001$).

§Significantly higher than the manufacturer-labeled output ($P < .0001$).

reduced by about one-half concentration⁵; that is, increasing the flow, pressure, or both so that the nebulizer delivers 0.9 mL/min increases the amount of methacholine delivered and therefore reduces the apparent PC₂₀. As a consequence, the standard method, which also produces less variability than the rebreathe method, has been recommended for calibrating nebulizers.^{5,6} However, only the rebreathe method can be used with the Digidoser because the system will not trigger the next activation if the subject does not exhale into the nebulizer.

We conclude that when using the KoKo Digidoser, methacholine PC₂₀ values of 8 mg/mL or greater should be interpreted with caution.

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REFERENCES

1. Ryan G, Dolovich MB, Obminski G, Cockcroft DW, Juniper E, Hargreave FE, et al. Standardization of inhalation provocation tests: influence of nebulizer output, particle size, and method of inhalation. *J Allergy Clin Immunol* 1981;67:156-61.
2. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing—1999. *Am J Respir Crit Care Med* 2000;161:309-29.
3. Statistical analysis system. Version 8. Cary (NC): SAS Institute; 2002.
4. Todd DC, Davis BE, Smyzniuk AJ, Cockcroft DW. Importance of dosimeter calibration method on nebulizer output. *Ann Allergy Asthma Immunol* 2005;94:45-7.

5. Davis BE, Todd DC, Cockcroft DW. Method of nebulizer calibration and methacholine. *J Allergy Clin Immunol* 2004;114:1474-5.

6. Hargreave F. Dosimeter: what is the dose? *Ann Allergy Asthma Immunol* 2005;94:14.

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Lipid transfer protein is involved in rhinoconjunctivitis and asthma produced by rice inhalation

To the Editor:

In spite of the fact that rice is one of the most consumed cereals worldwide, few cases of true rice allergy have been reported. Two different routes of exposure leading to immediate hypersensitivity reactions have been documented: ingestion of the cooked cereal¹ and inhalation of vapors during its boiling.² Besides a 33-kd glyoxalase I, the major rice seed allergens so far characterized belong to the 14-kd to 16-kd α -amylase inhibitor family of cereals.^{1,2} Wheat and rye members of this protein family are also involved in baker's asthma, caused by the occupational inhalation of these flours.³

Three patients with respiratory allergy to raw rice by inhalation were included in this study (Table I). All of them showed an acute allergic reaction (rhinoconjunctivitis or asthma) while they threw rice in different weddings. Two of them had a pollinic background, and all 3 patients were allergic to peach but tolerated rice by ingestion. Positive specific IgE determination (CAP system FEIA; Pharmacia Diagnostics, Uppsala, Sweden) and skin prick test (SPT) responses using commercial extracts (ALK-Abelló, Madrid, Spain) to rice and peach were obtained in all patients. SPTs, IgE immunodetection, and CAP inhibition assays were also performed using peach Pru p 3, which was isolated and quantified as previously reported,⁴ and a rice seed extract made in house. Such extract was obtained from defatted rice flour extracted with PBS buffer, and after centrifugation, the supernatant was dialyzed against H₂O and freeze-dried. Immunodetection with