

Comparison of non-invasive measures of cholinergic and allergic airway responsiveness in rats

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Abstract

Aim: Non-invasive analysis of tidal expiratory flow parameters such as Tme/TE (time needed to reach peak expiratory flow divided by total expiratory time) or midexpiratory tidal flow (EF₅₀) has been shown useful for phenotypic characterization of lung function in humans and animal models. In this study, we aimed to compare the utility of two non-invasive measures, EF₅₀ and Tme/TE, to monitor bronchoconstriction to inhaled cholinergic and allergic challenges in Brown-Norway rats.

Methods: Non-invasive measurements of Tme/TE and EF₅₀ were paralleled by invasive recordings of Tme/TE, EF₅₀ and pulmonary conductance (GL).

Results: First, dose-response studies with acetylcholine were performed in naive rats, showing that EF₅₀ better than Tme/TE reflected the dose-related changes as observed with the classical invasive outcome parameter GL. The subsequent determination of allergen-specific early airway responsiveness (EAR) showed that ovalbumin-sensitized and -challenged rats exhibited airway inflammation and allergen-specific EAR. Again, EF₅₀ was more sensitive than Tme/TE in detecting the allergen-specific EAR recorded with invasive and non-invasive lung function methods and agreed well with classical GL measurements.

Conclusion: We conclude that non-invasive assessment of EF₅₀ is significantly superior to Tme/TE and serves as a suitable and valid tool for phenotypic screening of cholinergic and allergic airway responsiveness in rats.

Keywords airway responsiveness, animal model, rat, respiratory function test, tidal expiratory flow.

Determination of pulmonary function in laboratory animals is an essential tool in many biomedical research areas including asthma. Animal models of experimental asthma have been important tools in providing useful information on the mechanisms underlying the pathophysiology of airway hyperresponsiveness (Drazen *et al.* 1999). Advantages of using the highly inbred Brown-Norway (BN) rat model to study respiratory mechanics in allergen-induced airway inflammation include its

well-developed airway smooth muscles, within-strain homogeneity of airway reactivity, and the development of allergen-specific airway responsiveness (AR) (Elwood *et al.* 1991, Xu *et al.* 1992).

The classical approach for precise and specific measurements of pulmonary mechanics is the invasive monitoring of pulmonary resistance (RL) or pulmonary conductance (GL). Recent advances in measuring respiratory function in intact, spontaneously breathing

animals, however, have enabled simple non-invasive routine analysis of pulmonary function in animal models (Pennock *et al.* 1979, Vijayaraghavan *et al.* 1994, Hamelmann *et al.* 1997, Peták *et al.* 1998). We have shown that midexpiratory tidal flow (EF₅₀), as measured by head-out body plethysmography, may be used as a non-invasive index of bronchoconstriction in rats (Glaab *et al.* 2002).

In the present study, we have for the first time evaluated an alternative non-invasive parameter of bronchoconstriction in BN rats. This composite index, Tme/TE, was originally described as an appropriate non-invasive surrogate parameter of bronchoconstriction in human adults and infants (Morris & Lane 1981, Clarke *et al.* 1994, Morris *et al.* 1995, Van der Ent *et al.* 1996). Tme/TE is defined as the time to reach maximum expiratory flow (Tme) divided by total expiratory time (TE). As normal post-inspiratory muscle braking is lost in airway obstruction, Tme/TE decreases. Whether Tme/TE might be more sensitive than EF₅₀ in detecting airways constriction in small animals is unknown. The primary objective of the present study was to compare the utility of non-invasive EF₅₀ vs. Tme/TE measurements as valid measures of AR in a well-established rat model of allergen-induced airway inflammation (Bellofiore & Martin 1988, Elwood *et al.* 1991). To this end, we performed dose-response studies with aerosolized ACh by non-invasive measurement of EF₅₀ and Tme/TE in conscious rats by head-out body plethysmography, followed by invasive monitoring of simultaneously measured Tme/TE, EF₅₀ and pulmonary conductance (GL) to inhaled cholinergic challenge in the same animals. Moreover, we determined directly the capability of these invasive and non-invasive indicators to detect early airway responsiveness (EAR) to ovalbumin allergen in allergic animals. Our findings show that the non-invasive measurement of EF₅₀ closely reflects the changes observed with invasively measured GL and EF₅₀ during bronchoconstriction to inhaled ACh and allergen and that EF₅₀ was superior to Tme/TE in detecting bronchoconstriction.

Materials and methods

Animals

Pathogen-free inbred male BN rats (Charles River, Sulzfeld, Germany), 6–8 weeks of age, were used in all experiments and were provided food and water *ad libitum*. Rats were divided into three main groups: (1) Cholinergic challenge with acetylcholine (ACh) ($n = 6$). After non-invasive monitoring of Tme/TE and EF₅₀, animals were reused for invasive determination of Tme/TE, EF₅₀ and GL to ACh challenge 24 h later. (2) Allergen-sensitized and -boosted rats for invasive ($n =$

6) and non-invasive ($n = 6$) measurements of EAR to inhaled ovalbumin (Ova). (3) Sham-treated controls for invasive ($n = 6$) and non-invasive ($n = 6$) measurements of EAR to inhaled Ova. All animal experimentation conformed to the guidelines of the 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (Council of Europe No. 123, Strasbourg, 1985).

Sensitization protocol

Allergic rats (allergic): On day 0, animals were sensitized by s.c. injection of 1 mg Ova (grade V; Sigma, Deisenhofen, Germany) precipitated in 20 mg of aluminium hydroxide gel in 0.25 mL normal saline ($n = 6$). An adjuvant consisting of 6×10^9 heat-killed Bordetella pertussis organisms (Chiron-Behring, Marburg, Germany) was injected i.p. at the same time. On days 7 and 14, the anaesthetized animals were boosted intratracheally via an orotracheal tube with 0.1 mL of 0.3% Ova in normal saline. Control rats (controls) were sensitized and boosted to normal saline instead of Ova ($n = 6$). This protocol was chosen to maximize the difference between allergic and control groups. On day 21, all animals were challenged with aerosolized Ova as described below.

Non-invasive measurement of AR in conscious rats

Airway responsiveness was assessed with a head-out body plethysmography system (model 855; HSE-Harvard, March-Hugstetten, Germany) as previously described (Vijayaraghavan *et al.* 1994, Glaab *et al.* 2002). Briefly, rats were placed in the body plethysmographs while the head of each animal protruded through a neck collar into the head exposure chamber, which was ventilated by a continuous airflow of 0.5 L min^{-1} . Rats held in head-out body plethysmographs tolerated non-invasive measurements very well. Monitoring of respiratory function was started when animals and individual measurements settled down to a stable level. For airflow measurement, a wire-mesh pneumotachometer and a differential pressure transducer coupled to an amplifier were attached in midposition to the side wall of each plethysmograph. For each animal, the amplified analog signal from the pressure transducer was digitized via an analog-to-digital converter. The pneumotachograph tidal flow signal was integrated with time to obtain tidal volume (VT). From these signals, all non-invasive parameters listed in Table 1 were calculated for each breath with a commercial software (HEM 3.5; Notocord, Paris, France).

Two non-invasive parameters were used as measures of bronchoconstriction: EF₅₀ calculates the tidal flow (mL s^{-1}) at the midpoint of expiratory tidal volume,

Table 1 Values for respiratory parameters at baseline

Respiratory parameters	Definition	Baseline values	
		Anaesthetized	Conscious
VT (mL)	Tidal volume	1.12 ± 0.14*	1.59 ± 0.13
<i>f</i> (breaths min ⁻¹)	Breathing frequency	86 ± 16*	114 ± 10
TE (s)	Time of expiration	0.33 ± 0.05	0.30 ± 0.03
Tme/TE (%)	Percentage expiratory time taken to reach tidal peak expiratory flow	20.5 ± 2.4*	39.1 ± 4.2
EF ₅₀ (mL s ⁻¹)	Midexpiratory tidal flow	8.74 ± 1.93	8.5 ± 0.7
GL (mL cmH ₂ O ⁻¹ s ⁻¹)	Pulmonary conductance	4.82 ± 0.89	–

Baseline values are mean ± SD obtained from 18 animals per group during a 5-min control period from conscious and anaesthetized, orotracheally intubated BN rats. In comparison with conscious rats, VT, Tme/TE and *f* values were significantly decreased in anaesthetized animals. **P* < 0.05 vs. conscious rats.

whereas Tme/TE (%) calculates the time to reach the maximum expiratory flow divided by total expiratory time. An example of EF₅₀ and Tme/TE patterns from conscious rats before and after challenge with aerosolized ACh is shown in Figure 1.

Invasive determination of AR

Airway responsiveness was assessed in anaesthetized and orotracheally intubated rats as reported previously

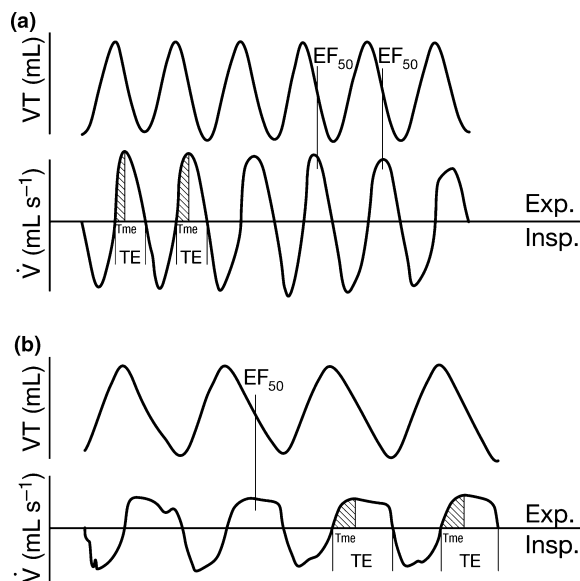


Figure 1 Characteristic modifications to the normal respiratory pattern in conscious rats. (a) Normal breathing pattern of BN rats. (b) Characteristic pattern of airway obstruction in response to inhaled ACh, illustrating the decline in both EF₅₀ and, to a lesser degree, in Tme/TE. (a) and (b), *top* tracings: Tidal volume signals. (a) and (b), *bottom* tracings: Corresponding tidal flow signals. A horizontal line separates inspiratory from expiratory tidal flows.

(Hoymann *et al.* 1994, Glaab *et al.* 2002, 2003). The spontaneously breathing animal was placed in supine position in a thermostat-controlled whole-body plethysmograph and anaesthesia was maintained with a gas mixture of 0.8% halothane in 30% oxygen. Tidal flow was determined by a wire-mesh pneumotachometer attached to the rear wall of the plethysmograph and connected to a differential pressure transducer. For measurement of oesophageal pressure, a water-filled polyvinyl chloride tube (13.5-cm length, 1-mm ID) was inserted into the lower third of the oesophagus and coupled to a pressure transducer. The amplified analog signal from the pressure transducer was digitized by an analog-to-digital converter. Pulmonary resistance (RL) was calculated from the integrated differences in oesophageal pressure and tidal flow. RL, Tme/TE, EF₅₀ together with all the other parameters listed in Table 1 were recorded simultaneously with a commercial software (HEM 3.5; Notocord). For better comparability of data, RL was expressed as GL (1/RL).

Challenge protocol

After recording of baseline values, AR to doubling doses of aerosolized ACh (ranging from 20 to 160 mg m⁻³; Sigma) in naive rats and AR to aerosolized Ova (grade V, 135 mg m⁻³) in sensitized and non-sensitized animals were assessed. Aerosols were generated by a computer-controlled aerosol generator system (Bronchy III, particle size 1.5–2 µm mass median aerodynamic diameter) as previously described (Hoymann *et al.* 1994, Glaab *et al.* 2002, 2003). For each animal, prechallenge baseline values of invasive and non-invasive respiratory parameters were determined as mean ± SD from all breaths collected during a baseline period of 5 min. This means that each animal served as its own control. The degree of AR to ACh and Ova

challenges was determined from minimum values of GL, Tme/TE and EF₅₀ and was expressed as per cent changes from corresponding baseline values.

Assessment of leucocyte distribution in bronchoalveolar lavage fluid

Bronchoalveolar lavage (BAL) was performed approximately 24 h after allergen challenge as previously described (Glaab *et al.* 2002).

Statistics

Values for all measurements were expressed as the mean $\pm$ standard deviation (SD). Differences between groups were analysed using Student's unpaired *t*-test. The mean values of the methods at different levels of cholinergic challenges were compared globally with Hotellings *T*²-test and the paired *t*-test for pairwise comparisons. All tests were performed two-sided at the level $\alpha = 0.05$.

Comparison of a new measurement technique with an established one is needed to see whether they agree sufficiently. A plot of the difference against the standard measurements will often appear to show a relation between difference and magnitude when there is none. A plot of the difference against the average of the standard and new measurements is unlikely to mislead in this way. Accordingly, the agreement between the methods was analysed by the method of Bland & Altman (1986, 1995). Graphically, the difference of each pair of measurement was plotted against their mean values. Agreement was expressed as the mean differences over all measurements and their corresponding 95% confidence intervals (95% CI). The limits of agreement were expressed as the mean differences $\pm$ 2 SD of the differences, together with their 95% CI. Differences of the per cent changes between two parameters within the 95% limits of agreement $\pm$ 25% were assumed acceptable. Statistics were performed with SPSS 12.

Results

Respiratory parameters in conscious and anaesthetized rats at baseline

To investigate the influence of anaesthesia on pulmonary function, respiratory parameters were recorded in anaesthetized and conscious BN rats at baseline. Table 1 illustrates the baseline values of respiratory variables obtained from conscious and anaesthetized rats. In contrast to constant EF₅₀ and TE values, there were significant differences in *f*, VT and Tme/TE values between anaesthetized and conscious animals at base-

line. We observed no differences in respiratory parameters between allergic and control rats at baseline when separated into conscious and anaesthetized groups.

Comparison of non-invasive and invasive measures of cholinergic AR in naive rats

To compare the utilities of EF₅₀ and Tme/TE, we first studied cholinergic AR to increasing doses of aerosolized ACh (20–160 mg m⁻³) in naive, conscious rats (*n* = 6) using non-invasive head-out body plethysmography. The same animals were reused for invasive measurement of cholinergic AR 24 h later.

The non-invasive measurements of EF₅₀ and Tme/TE to inhaled cholinergic challenge showed a dose-related decrease which was significantly higher for EF₅₀ compared with simultaneously measured Tme/TE at higher concentrations (*P* < 0.05, Fig. 2). After inhalation of each concentration of ACh, peak responses for EF₅₀ and Tme/TE were reached within 4 min and recovered to within 10% of the baseline during 1–5 min. To determine whether decreases in invasively or non-invasively monitored EF₅₀ and Tme/TE relate to each other or to changes in GL, we analysed the agreement between these measurements by the method of Bland and Altman. Agreement between EF₅₀ and Tme/TE was poor, the mean ranging from –11.4 to 26.8 (Table 2).

Subsequent invasive measurement of Tme/TE, GL and EF₅₀ to cholinergic challenge in anaesthetized, orotracheally intubated animals also showed a dose-dependent reduction in simultaneously recorded GL, Tme/TE and EF₅₀ values. Peak responses for GL, Tme/TE and EF₅₀ were reached within 1 min and recovered to within 10% of the baseline during 1–3.5 min. The magnitudes of cholinergic AR were significantly higher with GL and EF₅₀ compared with Tme/TE (*P* < 0.05).

As shown in Figure 2, invasive and non-invasive Tme/TE recordings were rather insensitive to reflect the dose-related bronchoconstrictive response to ACh exposures at higher concentrations. The following pairwise comparisons were significant: (at MCh 80 mg m⁻³) GL vs. Tme/TE_{an}, *P* $\leq$ 0.01; EF_{50con} vs. Tme/TE_{con}, EF_{50con} vs. Tme/TE_{an}, EF_{50con} vs. EF_{50an}, *P* < 0.05; (at MCh 160 mg m⁻³) GL vs. Tme/TE_{con}, GL vs. Tme/TE_{an}, GL vs. EF_{50an}, EF_{50con} vs. Tme/TE_{con}, EF_{50con} vs. Tme/TE_{an}, *P* $\leq$ 0.01; Tme/TE_{an} vs. EF_{50an}, Tme/TE_{an} vs. Tme/TE_{con}, *P* < 0.05.

As shown in Table 2, agreements between EF₅₀ and GL were acceptable, whereas poor agreement was found between Tme/TE and both GL and EF₅₀.

In comparison with simultaneously recorded GL, both surrogate parameters, EF₅₀ and Tme/TE, tended to underestimate the increase in bronchoconstriction by 6.5% (95% CI 3.3–9.7) and by 13.5% (95% CI 6.6–20.4), respectively.

Figure 2 Dose–response studies to cholinergic challenge (ACh aerosol 20–160 mg m⁻³) in naive rats. Values are means of per cent changes to baseline values, which were taken as 0%. Baseline values for each parameter were within the mean $\pm$ SD as listed in Table 1. Non-invasive measurement of cholinergic challenge was followed by invasive determination of simultaneously recorded EF₅₀, Tme/TE and GL to ACh exposure in the same animals ($n = 6$ each group) 24 h later. Invasive GL was the most sensitive parameter to detect dose-related cholinergic AR, followed by EF₅₀ recordings. Global Hotellings T^2 -test was positive. an, anaesthetized; con, conscious.

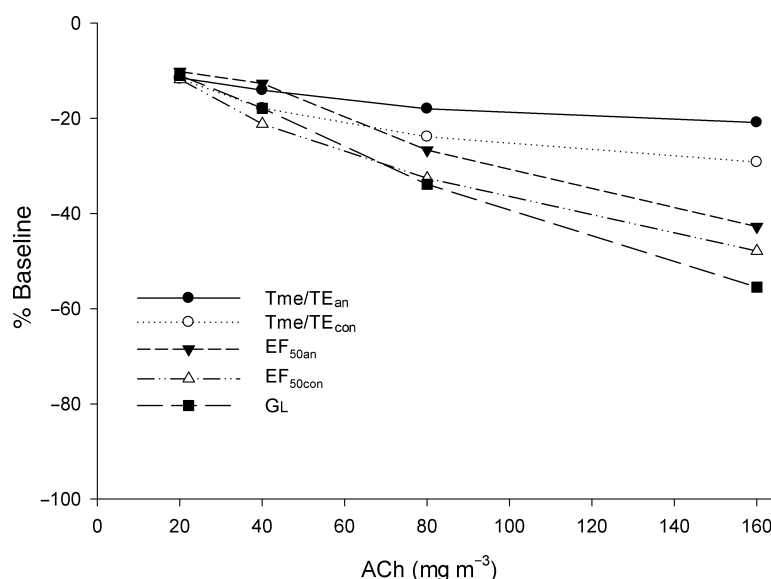


Table 2 Bland–Altman analysis of the differences in GL, EF₅₀ and Tme/TE

Parameters	Cholinergic AR		
	Mean	Upper limit	Lower limit
GL vs. EF _{50an}	6.5 (3.2 to 9.7)	21.9 (16.3 to 27.5)	-8.9 (-14.5 to -3.3)
GL vs. Tme/TE _{an}	13.5 (6.6 to 20.4)	46 (34.1 to 57.9)	-19 (-30.9 to -7.2)
GL vs. EF _{50con}	1.2 (-3.7 to 6.1)	24.4 (15.9 to 32.9)	-22 (-30.5 to -13.5)
GL vs. Tme/TE _{con}	8.9 (2.3 to 15.5)	40.2 (28.8 to 51.7)	-22.4 (-33.8 to -10.9)
EF _{50an} vs. EF _{50con}	5.3 (1.8 to 8.8)	21.8 (15.8 to 27.9)	-11.2 (-17.3 to -5.2)
EF _{50an} vs. Tme/TE _{an}	7 (1.9 to 12.1)	31 (22.2 to 39.8)	-17 (-25.8 to -8.2)
EF _{50con} vs. Tme/TE _{con}	7.7 (3.7 to 11.8)	26.8 (19.8 to 33.8)	-11.4 (-18.4 to -4.4)
Tme/TE _{con} vs. Tme/TE _{an}	4.6 (1.9 to 7.3)	17.4 (12.7 to 22.1)	-8.3 (-13 to -3.6)

Differences between GL, EF₅₀ and Tme/TE for naive BN rats during cholinergic AR. Values are mean (95% confidence intervals in brackets) for six animals per group. The upper and lower limits of agreement (mean $\pm$ 2 SD) as well as the corresponding 95% CI intervals (in brackets) are shown. an, anaesthetized rats; con, conscious rats.

Non-invasive EF₅₀ recordings differed from the GL method by only 1.2% (95% CI -3.7 to 6.1), whereas non-invasively measured Tme/TE differed from GL by 8.9% (95% CI 2.3–15.5%).

Reductions in EF₅₀, Tme/TE and GL values during invasive measurements were accompanied by small increases in f and decreasing values for VT, whereas TE remained nearly constant. With non-invasive measurements, EF₅₀ decreased with concomitant increases in TE and decreases in VT and f values.

Comparison of invasive and non-invasive measures of allergen-specific EAR

The allergen-specific EAR was investigated on day 21 in two groups divided into allergic and control groups (Fig. 3). To avoid unbalanced challenges with Ova,

each group was separated into two subgroups for invasive and non-invasive measurements of pulmonary function.

Invasive recordings in allergic rats showed significant reductions in simultaneously measured EF₅₀ and GL compared with control animals, indicating an Ova-specific EAR (GL: $P < 0.01$, EF₅₀: $P < 0.05$). As shown in Figure 3(a), the most prominent alteration was shown for GL with a reduction by $60.2 \pm 16\%$ ($P = 0.004$ vs. control) compared with a reduction by $42.9 \pm 7.3\%$ in EF₅₀ ($P = 0.013$ vs. control) and a small decrease by $-21 \pm 14.6\%$ in Tme/TE (non-significant vs. control).

Non-invasive measurements in allergic rats also demonstrated a marked allergen-specific EAR as manifested by a significant decline in EF₅₀ by $-48.7 \pm 6.2\%$ compared with a decrease by $21.5 \pm 9.2\%$ in control

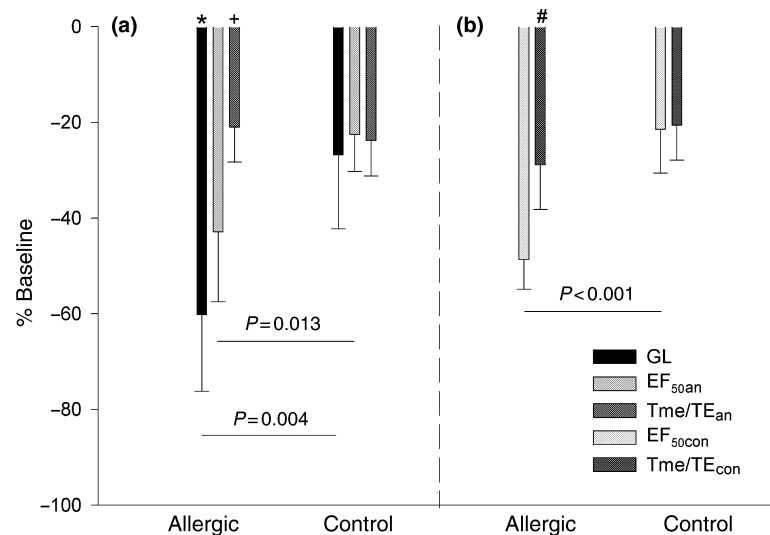


Figure 3 Values are mean (SD) of per cent changes to prechallenge baseline values, which were taken as 0%. Determination of Ova-specific EAR in allergic ($n = 6$) vs. control rats ($n = 6$ each group). To avoid a bias on results, EF₅₀ and Tme/TE responses were examined in separate groups of invasively and non-invasively measured rats. (a) Invasive measurement of pulmonary function in anaesthetized, orotracheally intubated rats. (b) Non-invasive measurement of pulmonary function in conscious animals. Allergic rats exhibited a pronounced Ova-specific EAR relative to controls, as shown by significant reductions in EF₅₀ (invasive and non-invasive) and GL measurements. The invasive and non-invasive measurements of Tme/TE failed to reflect the allergen-specific EAR in allergic animals. *GL vs. EF₅₀, GL vs. Tme/TE, $P < 0.01$; +EF₅₀ vs. Tme/TE, $P < 0.05$; #EF₅₀ vs. Tme/TE, $P < 0.01$.

animals ($P < 0.001$, Fig. 3b). The magnitude of the response was similar to the decline observed with invasively recorded EF₅₀. By contrast, allergic challenge resulted in a mild non-significant decline in Tme/TE relative to controls, suggesting that the sensitivity of the Tme/TE method was markedly less pronounced compared with EF₅₀ recordings.

Allergic airway inflammation

The allergic animals showed significant increases in eosinophils, lymphocytes, neutrophils and macrophages in BAL fluid (Table 3) over controls, indicating the presence of an inflammatory response in the lungs of allergic rats.

Table 3 Leucocyte distribution of BAL fluid

	Control	Allergic
Macrophages, $\times 10^6$	4.6 ± 1.1	$12 \pm 4.5^*$
Eosinophils, $\times 10^6$	0.2 ± 0.1	$3.1 \pm 2^*$
Lymphocytes, $\times 10^6$	0.1 ± 0.1	$0.4 \pm 0.3^{**}$
Neutrophils, $\times 10^6$	0.1 ± 0.1	$0.9 \pm 0.5^*$

Values are mean $\pm$ SD from 12 animals per group (pooled data from invasive and non-invasive measurements). Macrophages, lymphocytes, neutrophils and eosinophils recovered from bronchoalveolar lavage (BAL) fluid 24 h after Ova aerosol exposure were significantly higher in the allergic animals.

* $P < 0.001$ vs. control; ** $P < 0.01$ vs. control.

Discussion

In this study, we compared the utility of two non-invasive parameters, Tme/TE vs. EF₅₀, to measure AR in response to cholinergic challenge followed by allergen exposure in the same, spontaneously breathing rats. Our results show that EF₅₀ was more sensitive than Tme/TE in reflecting the airway responses to ACh and allergen observed with the classical invasive parameter GL.

Recent progresses in rat genetic technology have opened up new opportunities to develop better rat models of allergic asthma which would be helpful in the development of new therapeutics (Rat Genome Sequencing Project Consortium 2004, Mashimo *et al.* 2005). For a proper phenotypic characterization of AR in experimental models, however, it is critically important to measure pulmonary function as reliably as possible.

With the recent emphasis on the benefits of non-invasive technology, there has been renewed interest in analysing tidal flow patterns as a tool in the assessment of airway obstruction (Morris & Lane 1981, Vijayaraghavan *et al.* 1993, Calverley & Koulouris 2005). We have previously described the non-invasive measurement of midexpiratory flow (EF₅₀) by head-out body plethysmography as a suitable and valid instrument to measure AR in several conscious animals at a time. In the current report, we have for the first time assessed another index of airway obstruction derived from the analysis of tidal expiratory flow. This composite index,

Tme/TE, has been shown useful to indicate airway obstruction in young children, in cases where forced expiratory manoeuvres were unreliable or inapplicable (Clarke *et al.* 1994, Stocks *et al.* 1994, Banovcin *et al.* 1995, Van der Ent *et al.* 1996). Therefore, application of this human-derived parameter of bronchoconstriction seemed particularly attractive for use in an experimental animal model.

The values for basic respiratory parameters measured from both conscious and anaesthetized BN rats were reproducible and quite similar to those reported previously for this strain (Table 1) (Watanabe *et al.* 1995, Glaab *et al.* 2002).

To assess and compare the utilities of EF₅₀ and Tme/TE as appropriate measures of AR, we conducted dose–response measurements to inhaled ACh in both naive conscious and anaesthetized animals. Non-invasive monitoring of pulmonary function showed that EF₅₀ appropriately reflected the AR to increasing doses of ACh as observed with GL as an outcome indicator. Analysis of non-invasive determination of Tme/TE and EF₅₀ demonstrated that EF₅₀ was significantly superior to Tme/TE in detecting dose-related cholinergic AR.

Direct comparison of invasively recorded EF₅₀ and GL with non-invasive measurement of EF₅₀ revealed that GL agreed closely with invasive and non-invasive EF₅₀ recordings and showed comparable decreases of the ACh dose–response curve over baseline values. By contrast, invasively and non-invasively measured Tme/TE failed to reflect the dose-related changes observed with GL and EF₅₀ recordings.

In accordance with previous studies, we found that allergic rats demonstrated an Ova-specific EAR. Detection of EAR was based on corresponding decreases in GL, Tme/TE and EF₅₀, respectively. Demonstration of allergen-specific EAR in allergic rats was linked with a pronounced influx of leukocyte subsets in BAL fluid.

In response to specific allergen challenge, invasive and non-invasive Tme/TE measurements largely underestimated the degree of bronchoconstriction as compared with GL and EF₅₀ monitoring. Likewise, BN rats displayed no characteristic pattern of expiratory flow as seen in patients with airflow obstruction that have shown a rapid rise to peak flow (Tme) (Morris & Lane 1981, Morris *et al.* 1995). It should also be noted in this context that the ratio Tme/TE might reflect the neuromuscular effects on the total respiratory system more than the true function of the airways (Banovcin *et al.* 1995). By contrast, non-invasively monitored EF₅₀ was sufficiently sensitive in detecting the degree of cholinergic and allergen-specific AR compared with invasive GL recordings. The current study is consistent with previous investigations reporting that EF₅₀ tends to slightly underestimate the real amount of AR as measured with traditional invasive measures of lung mechanics (Glaab

et al. 2002). A possible explanation for this discrepancy might be that GL rather than EF₅₀ reflects changes in tissue resistance, known to largely contribute to the overall resistance to nebulized bronchoconstrictors in rats (Nagase *et al.* 1994, Hall *et al.* 1999).

The non-invasive measurements of EF₅₀ may also be partly affected by altered upper airway resistance. Because of the rapid onset and resolution of the response, however, it seems unlikely that oedema or mucus hypersecretion in these upper airways were responsible for the increased AR.

Another issue is that delivery of aerosols directly into the lungs via an orotracheal tube results in pronounced peripheral aerosol deposition. In conscious animals, however, there will be substantial deposition in the nasal passages and upper airways, which should contribute to the observed differences with non-invasive EF₅₀ measurements. Despite these limitations and even though the calculated inhalation doses for ACh and Ova in conscious rats may not be as accurate as in intubated rats, the observed EF₅₀ responses still reflected the enhanced AR to ACh and allergen.

The principal advantages of the non-invasive EF₅₀ method over commonly used non-invasive methods such as whole-body plethysmography are that it is based on physiological principles, has a physical meaning thus enabling quantitative interpretation of data between animals, and shows no tendency to overestimate the amount of AR when directly compared with GL measurements (Peták *et al.* 2001, Hantos & Brusasco 2002).

In summary, we have shown that the non-invasive EF₅₀ method was superior to the non-invasive measurement of Tme/TE in reflecting AR to inhaled ACh and allergen in BN rats. We conclude that the non-invasive EF₅₀ method is directly related to lung resistance and seems particularly appropriate for rapid and repeatable screening of airway function in large numbers of animals.

Conflict of interest

The authors declare that they have no competing interests.

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