# Faster Rise of Exhaled Breath Temperature in Asthma
## A Novel Marker of Airway Inflammation?

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**In asthma there is increased vascularity of the airway mucosa, altering heat loss in the airways. We hypothesized that as a result of these inflammatory changes, asthmatic patients would have elevated rates of the exhaled air temperature increase ($\Delta e^{\circ}T$). We measured $\Delta e^{\circ}T$ in 18 asthmatic subjects (mean age ± SEM, 38 ± 8 yr; 9 male, $FEV_1$ 74 ± 10%) and 16 normal volunteers (mean age ± SEM, 33 ± 3 yr) and compared it with exhaled nitric oxide (NO) as a marker of inflammation. $\Delta e^{\circ}T$ was measured during a flow- and pressure-controlled single exhalation with a fast response (1 ms) thermometer. The end-expiratory plateau temperature was similar in asthmatic compared with normal subjects (35.75 ± 0.6° C and 34.45 ± 0.8° C, $p > 0.05$). However, $\Delta e^{\circ}T$ was greater in asthmatic subjects (8.17 ± 0.83° C/s and 4.12 ± 0.41° C/s, $p < 0.01$) and correlated with NO ($r = 0.65$, $p = 0.034$). $\Delta e^{\circ}T$ was increased in normal subjects (from 4.28 ± 0.8° C/s to 7.60 ± 0.5° C/s, $p < 0.01$) but not in asthmatic patients (from 8.28 ± 0.41° C/s to 8.80 ± 0.41° C/s, $p > 0.05$) after the inhalation of albuterol, indicating that $\Delta e^{\circ}T$ may reflect bronchial blood flow. Asthmatic subjects have elevated $\Delta e^{\circ}T$. This may represent a novel, noninvasive means of measuring airway blood flow and inflammation in asthma.**

**Keywords:** temperature; asthma; nitric oxide; inflammation

Asthma is an inflammatory disease of the airways. The histologic examination of the bronchial wall has revealed increased vascularity (1–3) and bronchial blood flow (4). These changes contribute to the regulation of airway temperature and tone (5). Reduction in airway temperature can trigger bronchoconstriction which can be reduced by decreasing mucosal blood supply with norepinephrine (5), indicating that mucosal blood flow and airway temperature are strictly associated. That airway temperature plays a role in the physiology of asthma was confirmed by the observation that the airways of asthmatic patients rewarm more rapidly than normal after hyperventilation and exercise (6). This finding suggests the possibility that hyperemia in the walls of the tracheobronchial tree may contribute to the bronchial narrowing and faster rewarming of exhaled breath.

Several inflammatory mediators known to be released in asthma may contribute to bronchial vascular dilation. Potential candidates include histamine, bradykinin (7), leukotrienes (8), platelet-activating factor (PAF) (9), prostaglandin $E_2$, adenosine (10), nitric oxide (NO) (11, 12), and mediators released by autonomic sensory nerves (13). Therefore, there are several inflammatory mediators capable of causing bronchial vascular dilation and changes in airway temperature.

NO is a gas produced by several types of pulmonary cells, including inflammatory, endothelial, and airway epithelial cells. NO can be measured in the exhaled breath (14). Elevated levels of exhaled NO in asthma (14, 15) are likely to be due to the activation of the inducible form of NO synthase (iNOS) by inflammatory cytokines (16) and therefore, reflect airway inflammation. It is noteworthy that NO plays an important role in regulating bronchial vascular tone (11) and increasing bronchial blood flow (11, 12). We hypothesized that patients with asthma would have higher exhaled breath temperatures compared with normal subjects because of higher concentrations of NO and other vasodilating mediators in the airways, resulting in increased bronchial blood flow. In addition, to investigate the correlation between exhaled breath temperature and bronchial blood flow, we studied the effect of the inhalation of a vasodilator, albuterol, on exhaled breath temperature.

We developed a simple and reproducible method for the measurement of exhaled breath temperature. Because NO causes bronchial vasodilation (17), we also investigated whether there was a correlation between concentrations of exhaled NO and breath temperature.

## METHODS

### Patients

Eighteen patients (9 male, 9 female, age 38 ± 8 yr, $FEV_1$ 74 ± 10% of predicted; 9 on inhaled steroid treatment were studied, 6 patients had moderate persistent asthma, 3 mild persistent asthma, and 9 mild intermittent asthma), and 16 control subjects (age 33 ± 3 yr, 8 male, 8 female) were recruited from our outpatient clinic and from volunteers (Table 1). Review of medical records confirmed that the diagnosis of asthma was established in each patient according to American Thoracic Society criteria (18). Patients with acute chest infection, upper respiratory tract infection, or disease exacerbation during the month before enrollment were excluded from the study. Patients with history of diabetes, liver disease, heart failure, lung cancer, or alcohol or drug abuse were not eligible for the study. All subjects were lifelong nonsmokers. All subjects had at least 1 h of rest before gas and exhaled breath temperature measurement, to eliminate the effect of any possible exposure to high ambient gas concentrations during their journey to the hospital as well as the effect of exercise-induced changes of airway temperature. Lung function tests were performed after exhaled breath analysis for NO. All asthmatic patients refrained from using $\beta_2$ agonists and corticosteroids for at least 12 h before the study. The tests were carried out in an air-conditioned room where the ambient temperature was maintained between 23 and 25° C and the ambient humidity 60% and 65%.

### Study Design

The study was approved by the Brompton & National Heart and Lung Institute (NHLI) Ethics Committee (Reference 00-132). All the subjects gave informed consent.

Patients were examined in the morning. After a full clinical examination, exhaled breath temperature and exhaled NO were measured after at least 1 h of rest in the laboratory. This was followed by spirometry. In five normal subjects and five asthmatic patients se-

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**TABLE 1. PATIENT CHARACTERISTICS***

| | Asthma Steroids ($n = 9$) | Asthma No Steroids ($n = 9$) | Normal ($n = 16$) |
|---|---|---|---|
| Age, yr | 41 ± 8 | 35 ± 8 | 33 ± 3 |
| Sex, M/F | 4/5 | 5/4 | 8/8 |
| $FEV_1$, % predicted | 74 ± 7 | 74 ± 10 | 95 ± 9 |
| RV/TLC, % predicted | 106 ± 9 | 98 ± 7 | 96 ± 9 |
| Therapy | | | |
| Inhaled β-adrenergics | 9 | 9 | 0 |
| Inhaled corticosteroids | 9 | 0 | 0 |

*Definition of abbreviation*: RV = residual volume.
* Values are means ± SEM.

lected randomly from those willing to participate, the measurement of exhaled breath temperature was repeated 10 min after the inhalation of 200 μg of albuterol from a metered-dose inhaler.

### Exhaled Breath Temperature Measurement

During a flow- and pressure-controlled exhalation (exhalation flow rate 10 to 11 L/min, mouth pressure 10 cm $H_2O$) from total lung capacity through a 2.77-mm mouthpiece (19), exhaled breath temperature was measured by a fast-response (1 ms) high-accuracy (0.015 ± 0.027° C) thermometer (Picotech Ltd, Cambridge, UK) interfaced with a computer by a single-channel Picotech oscilloscope (model ADC 42, resolution 12 bits) allowing online recording of exhaled breath temperature.

In a preliminary study, exhaled breath temperature tracings were analyzed mathematically. The tracings proved to have an exponential rise, and the point at 63% of the total temperature increase was chosen to study the slope of the curves because it represents two time constants of the maximal °T change and therefore allows a better mathematical characterization of the tracings before plateau. The rate of temperature increase (Δe°T) calculated between the beginning of exhalation and 63% of the total temperature increase (a/b, where "a" is 63% of Δ°T and "b" the time to reach "a", Figure 1) proved to be the more reproducible parameter to characterize the curves. We investigated the intrasession variability of the method (5-min interval) to verify the possible changes of exhaled breath temperature owing to the reproducibility of the method itself. We also studied the 1-d variability of the method to evaluate the biologic changes of exhaled breath temperature. Both the intrasession and intersession variability were satisfactory. *See* the online data supplement for details regarding the reproducibility of the method.

### Exhaled NO Measurements

Exhaled NO was measured using a modified chemiluminescence analyzer (model LR2000; Logan Research, Rochester, UK), sensitive to NO from 1 to 5,000 parts per billion (ppb) (by volume), and with a resolution of 0.3 ppb, which was designed for online recording of exhaled NO concentration. The analyzer was calibrated using certified NO mixtures (90 ppb and 436 ppb) in nitrogen (BOC Special Gases, Guildford, UK). Measurements of exhaled NO were made by slow exhalation (5 to 6 L/min) from total lung capacity for 20 to 30 s against a resistance (3 ± 0.4 mm Hg) (20).

### Lung Function Tests

After the measurement of exhaled gases, all patients underwent pulmonary function tests (PFT), including spirometry and lung volumes, using a Jaeger Master Lab Compact Transfer (Erich Jaeger Ltd., Leicestershire, U.K.).

### Statistics

GraphPad Prism package Version 3 and SPSS version 9 (San Diego, CA) were used for statistical analysis. Comparisons between groups were made by one-way analysis of variance (ANOVA) with Bonferroni's correction for multiple comparisons. Levene's test was used to confirm equal variances (SPSS package). The *t* test was used for pairwise comparisons. Data were expressed as means ± SEM. Significance was defined as a p value of $< 0.05$. The data were normally distributed. The relationship between the exhaled breath temperature, NO, and $FEV_1$ was tested with the linear correlation coefficient.

## RESULTS

### Exhaled Air Temperature

The end-expiratory plateau temperature was similar in asthmatic compared with normal subjects (35.75 ± 0.6° C and 34.45 ± 0.8° C, $p > 0.05$).

Δe°T was higher in asthmatic patients (8.17 ± 0.83 Δ° C/s) compared with normal subjects (4.12 ± 0.41 Δ°C/s, $p < 0.01$, Figure 2). Δe°T was elevated to a similar extent in patients with mild persistent and moderate asthma (on inhaled corticosteroids and $\beta_2$ agonists) (8.37 ± 0.96 Δ° C/s) and patients with mild intermittent asthma (on $\beta_2$ agonists as needed only) (7.96 ± 1.4 Δ° C/s, $p > 0.05$).

In five normal volunteers Δe°T was increased after the inhalation of 200 μg of albuterol (4.28 ± 0.41 Δ° C/s and 7.6 ± 0.6 Δ° C/s, $p < 0.01$), whereas this effect was not present in asthmatic patients (8.28 ± 0.41 Δ°C/s and 8.80 ± 0.41 Δ° C/s, $p > 0.05$, $n = 5$, Figure 3).

Δe°T was weakly correlated with airway obstruction as assessed by $FEV_1$ ($r = -0.47$, $p = 0.045$, Figure 4A).

### Exhaled NO

NO levels were similarly elevated in steroid-naive (15.0 ± 6.2 ppb) and steroid-treated patients (11.98 ± 3.1 ppb, $p > 0.05$, $F < 0.05$) compared with the control group (6.7 ± 0.5 ppb, $p <$

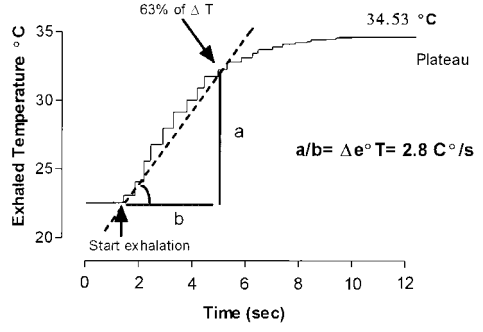


***Figure 1.*** Online recording of exhaled breath temperature of a normal subject. "*a*" represents the increase of temperature from baseline to 63% (two time constants) of the maximal increase, and "*b*" is the time required to reach it. *a/b* is the slope of the curve and represents the rate of temperature increase from baseline to 63% of the total temperature increase (Δe°T).

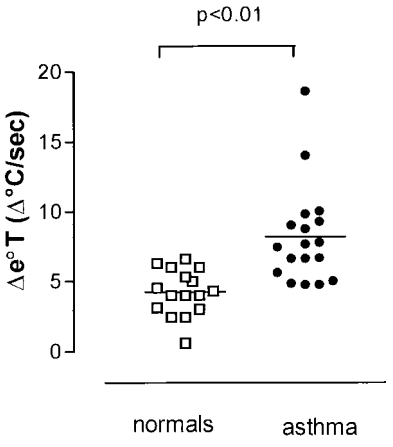


***Figure 2.*** Levels of exhaled breath temperature increase (Δe°T) in normal subjects (*open squares*) and patients with asthma (*filled circles*).

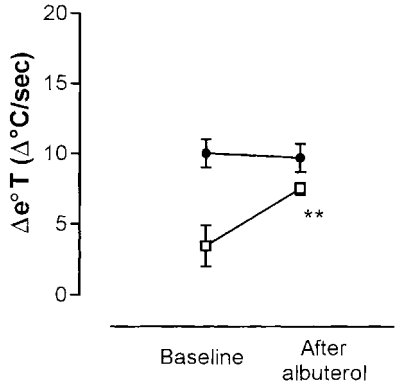


*Figure 3.* Changes of exhaled breath temperature increase (Δe°T) in normal subjects (*open squares*) and asthmatic patients (*filled circles*) after inhalation of 200 μg of albuterol. Mean Δe°T ± standard error of mean are shown (n = 5). **p < 0.01.

0.05, F > 0.05) and were positively correlated with the concentrations of exhaled Δe°T (r = 0.65, p = 0.034, Figure 4B).

## DISCUSSION

We have found that patients with asthma have faster increases of exhaled breath temperature during a single breath exhalation compared with normal subjects and that this is correlated to the concentration of exhaled NO. We interpret these data as confirmation that asthmatic patients have impaired heat exchange in the airways, and we hypothesize that airway inflammation and a high concentration of NO may cause vasodilation of the bronchial circulation, contributing to increased heat exchange.

Changes in bronchial blood flow can alter airway responsiveness and airway temperature (5), confirming that the bronchial circulation may control airstream temperature and contribute to airway narrowing. That bronchial blood flow and airway temperature are associated is indicated by the finding that temperature changes can induce bronchoconstriction and that this can be prevented by reducing mucosal blood supply using inhaled vasoconstrictors (5). Considering that airway temperature may reflect bronchial blood perfusion (21) and plays a role in bronchoconstriction, we developed a novel noninvasive method for the measurement of exhaled breath temperature and applied it to a group of patients with asthma and normal volunteers.

Patients with asthma had a significantly faster rise of breath temperature during exhalation compared with normal subjects. We presume that this is due to the increased vascularity of the bronchial vessels (2) and elevated blood supply (4) and therefore heat transfer across the bronchial wall. Hyperemia and hyperperfusion are a consistent feature of tissue inflammation; therefore, the finding of increased exhaled air temperature in asthmatic patients may be due to increased blood flow.

Even though we did not directly measure bronchial blood flow in the present study, the hypothesis that exhaled breath temperature reflects bronchial blood flow is supported by the finding that the inhalation of albuterol, a vasodilator (22), induces an increase of Δe°T in normal volunteers. On the contrary, in asthmatic patients, as previously shown for bronchial blood flow (4, 23), there were no significant changes of airway temperature after the inhalation of albuterol. The airway vessels may be already maximally vasodilated because of tissue inflammation and release of inflammatory compounds, and albuterol may not be able to further increase their diameter. Thus, we hypothesize that there might be a maximal increase in bronchial blood flow which is reached in asthmatics and prevents a further increase with inhaled vasoactive compounds such as albuterol. Further studies are necessary to investigate this hypothesis. The use of albuterol was preferred in this study to the use of a decongestant such as methoxamine because of the simplicity of administration and experience with the drug. The choice was also based on the knowledge that the inhalation of albuterol induces bronchial vasodilation 10 min after inhalation in normal subjects.

In this cross-sectional study we could not show differences of Δe°T in corticosteroid-treated compared with untreated asthmatic patients, despite the efficacy of steroids in reducing bronchial blood flow (23). We suggest that the vasoconstrictive action of inhaled corticosteroids may have been balanced by $\beta_2$-induced vasodilation, resulting in minimal changes in bronchial artery diameter and blood flow and therefore no net changes of Δe°T. Further placebo-controlled studies are necessary to investigate the acute action and the time course of inhaled steroids on Δe°T also considering that patients on chronic corticosteroid treatment may have their vascular sensitivity to albuterol partially restored (23).

There was a weak negative correlation between the rate of increase of exhaled breath temperature and bronchonconstriction as assessed by $FEV_1$. The vascular engorgement of airway vessels and consequent thickening of the mucosa, leads to narrowing of the lumen of the bronchi, an increase in airway resistance, and a decrease in forced expiratory rates (24). If thoracic blood volume is rapidly increased by shifting blood from the legs via antishock trousers, the size of the obstructive response is amplified (25). Such an effect is also seen with vascular volume expansion with intravenous saline (26). A reduction in airway caliber caused by increased vascularity and bronchial blood flow may explain the negative correlation between the rate of exhaled breath temperature increase and $FEV_1$. This hypothesis is further supported by the finding that patients with severe asthma have increased airway vascularity compared with patients with mild or moderate asthma (1, 2, 27).

NO is a gas produced by several types of pulmonary cells, including inflammatory, endothelial, and airway epithelial cells. Elevated levels of exhaled NO in asthma (14), and interstitial lung disease (28) are likely to be due to the activation of iNOS and therefore may reflect airway inflammation. In addition,

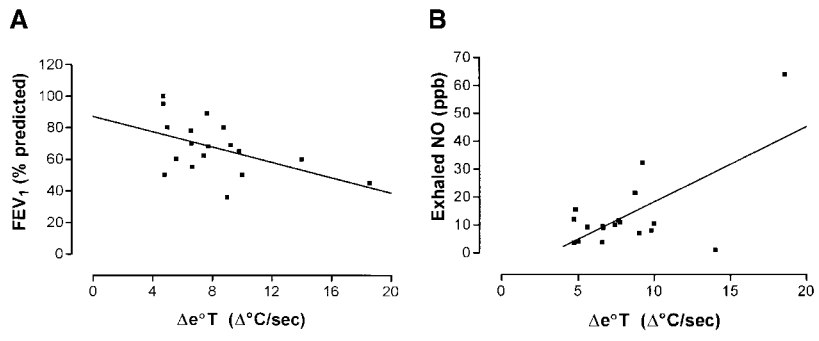


*Figure 4.* Correlation of exhaled breath temperature increase (Δe°T) with $FEV_1$ (*A*) and NO (*B*) in patients with asthma. (*A*) r = −0.47; p = 0.045; (*B*) r = 0.65; p = 0.034.

the activity of iNOS, the inducible enzyme responsible for the synthesis of NO, is temperature-dependent (29), therefore elevated airway temperatures in patients with asthma may induce further synthesis of NO. NO is a potent vasodilator and plays a major role in the regulation of bronchial vasomotor tone (17), so that elevated levels of NO may lead to vasodilation, increased bronchial blood flow, and exhaled breath temperature. Further studies evaluating the effect of inhibitors of NO synthesis are necessary to confirm this hypothesis.

The cardinal signs of inflammation are rubor (redness), calor (heat), tumor (swelling), dolor (pain), and impaired function. Exhaled breath temperature and bronchial blood flow may reflect rubor and calor in the airways and therefore may be markers of tissue inflammation; this is confirmed by the positive correlation between $\Delta e^{\circ}T$ and exhaled NO. Hyperemia and tissue temperature may be the final common pathways of inflammation; the measurement of these parameters may be another noninvasive way to assess the level of inflammation in the airways.

Measurement of exhaled breath temperature may be another means of detecting and monitoring cytokine- and oxidant-mediated inflammation and of assessing anti-inflammatory treatments. Further studies are necessary to investigate the correlation of these new measurements with other markers of inflammation in exhaled breath condensate and induced sputum, and their clinical utility in the follow-up of patients with asthma.

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