

DIAGNOSTICS

Exhaled breath temperature in children: reproducibility and influencing factorsS. Vermeulen, MD¹, M. Barreto, MD², F. La Penna, MD², A. Prete, MD², S. Martella, L Tech², F. Biagiarelli, MD², and M. P. Villa, MD²¹Faculty of Medical Sciences, University of Groningen, Groningen, The Netherlands and ²NESMOS Department, Pediatric Unit Sant'Andrea Hospital, Faculty of Medicine and Psychology, University "La Sapienza", Rome, Italy**Abstract**

Objective: This study will investigate the reproducibility and influencing factors of exhaled breath temperature measured with the tidal breathing technique in asthmatic patients and healthy children. **Methods:** Exhaled breath temperature, fractional exhaled nitric oxide, and spirometry were assessed in 124 children (63 healthy and 61 asthmatic), aged 11.2 ± 2.5 year, M/F 73/51. A modified version of the American Thoracic Society questionnaire on the child's present and past respiratory history was obtained from parents. Parents were also asked to provide detailed information on their child's medication use during the previous 4 weeks. Ear temperature, ambient temperature, and relative-ambient humidity were also recorded. **Results:** Exhaled breath temperature measurements were highly reproducible; the second measurement was higher than the first measurement, consistent with a test–retest situation. In 13 subjects, between-session within-day reproducibility of exhaled breath temperature was still high. Exhaled breath temperature increased with age and relative-ambient humidity. Exhaled breath temperature was comparable in healthy and asthmatic children; when adjusted for potential confounders (i.e. ambient conditions and subject characteristics), thermal values of asthmatic patients exceeded those of the healthy children by 1.1°C . Normalized exhaled breath temperature, by subtracting ambient temperature, was lower in asthmatic patients treated with inhaled corticosteroids than in those who were corticosteroid-naïve. **Conclusion:** Measurements of exhaled breath temperature are highly reproducible, yet influenced by several factors. Corrected values, i.e. normalized exhaled breath temperature, could help us to assess the effect of therapy with inhaled corticosteroids. More studies are needed to improve the usefulness of the exhaled breath temperature measured with the tidal breathing technique in children.

Keywords

Asthma, children, exhaled breath temperature, exhaled nitric oxide, inflammation, lung function

History

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Introduction

Airway inflammation is an important aspect in the pathogenesis of asthma. An invasive assessment of airway inflammation with bronchoscopy (lavage and biopsy) or by a sputum analysis of eosinophilia is not feasible as a routine asthma control test [1–3]. Several non-invasive markers of airway inflammation are now being investigated; these markers can be implemented in a routine asthma control visit and are easily measured in children. Currently, the most widely used marker is the fractional concentration of exhaled nitric oxide (FE_{NO}), a marker of atopic-eosinophilic inflammation [4].

Recent studies hypothesize that the measurement of the exhaled breath temperature (EBT) could be used as a non-invasive marker for evaluating airway inflammation in

asthmatic patients [5,6]. Inflammation is defined by five cardinal clinical signs, i.e. rubor (redness and hyperaemia), calor (heat), dolor (pain), tumor (swelling), and functio laesa (impaired function). Histological examinations of the bronchial airway walls have demonstrated increased vascularization, while a soluble inert gas uptake method showed increased bronchial blood flow in asthmatic patients [7,8]. Changes in EBT correlate with changes in bronchial blood flow and FE_{NO} in asthmatic patients [9]. Consequently, it has been proposed that EBT and bronchial blood flow may reflect calor and rubor and, therefore, may act as markers for airway inflammation and vascular remodeling in these patients [9].

Several methods for measuring EBT, such as the rate of EBT increase ($\Delta e^\circ T$), the peak of expiratory temperature (PET) and the plateau value at the end of expiration (PLET) have been proposed [5,6,9–11]. The techniques and devices used for assessing $\Delta e^\circ T$, PET and PLET are not easy to use; they require cooperation from the patients, because the measurements involve a single vital capacity maneuver. Popov et al. have introduced a new and simplified device for measuring EBT that consists of a thermally isolated

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chamber which contains a copper tube with a thermal sensor. This thermal sensor records the exhaled air temperature until a steady state is reached. The device does not require a special breathing technique; the subject inhales through the nose and exhales through the mouth, consistent with tidal breathing [12,13]. Measurements with this new device in adult subjects have been found reproducible, not influenced by ambient conditions and useful to distinguish uncontrolled asthmatic patients from healthy subjects [12]. These findings, however, cannot be extrapolated to the pediatric population. In addition, age-related physiological changes and subject's cooperation could influence tidal breathing EBT measurements in children.

In this study, we investigated the reproducibility and influencing factors of EBT measurements in a group of asthmatic patients and healthy children. We also sought whether this new practical instrument is suitable for assessing differences in EBT according to the disease state or therapy with inhaled corticosteroids (ICs).

Materials and methods

Study population

Subjects were recruited from July 2010 until July 2011. Asthmatic children attending our outpatient clinic for respiratory diseases had fully controlled or partially controlled asthma and were diagnosed in accordance with the Global Initiative for Asthma guidelines [1]. They had previously documented bronchial hyper-responsiveness, as measured by either the bronchial response to Salbutamol (FEV_1 increase >12% of predicted) or a positive response to exercise testing (FEV_1 fall >12% of baseline). Schoolchildren (Sant'Orsola School, Rome) and healthy outpatients attending our pediatric unit were recruited to form the control group. Healthy children were defined as non-atopic (i.e. a negative personal or parental history for atopic diseases), without a history of chronic respiratory symptoms and having a normal spirometry. Medication use during the 4 weeks preceding the measurements, including inhaled corticosteroids (ICs) and rescue medication with inhaled β_2 -sympathomimetics, was recorded. Subjects suffering acute respiratory illnesses less than 4 weeks before enrolment were excluded from the study.

Ethics statement

This study was approved by the Medical Ethics Committee of the Sant'Andrea Hospital in Rome. A written informed consent from all parents was obtained. Children who were 12 years or older also provided written informed consent, while children who were younger than 12 years of age provided a verbal informed consent.

Study design

We recruited a population able to cooperate with lung function and non-invasive measurements of airway inflammation (as measured by FE_{NO}), because the variability of EBT measurements is expected to increase with decreasing age. Measurements included EBT, FE_{NO} , and spirometry (in this order) in all children. Skin prick testing was

performed in asthmatic patients only. All measurements were performed during the same morning, between 8:30 and 9:30 AM. To assess short-term (within-day) reproducibility, a subgroup of children repeated EBT measurements in a second session, in the afternoon, between 2:30 and 3:30 PM. This subgroup consists of 13 subjects. Ten schoolchildren were randomly selected to perform the afternoon measurements. We also received consent from three asthmatic patients to be tested again in the afternoon. A physician unaware of questionnaire results was assigned to perform the selection procedure. Reproducibility of EBT measurements was assessed in two different ways:

1. Within-session measurements (in duplicate).
2. Between-session within-day measurements (morning vs. afternoon).

Within-session reproducibility was assessed in the total population. Both within-session and between-session reproducibility were assessed in the subgroup of 13 subjects which performed morning and afternoon measurements.

Both EBT and its normalized value, obtained by subtracting the ambient temperature from EBT (nEBT) [6], were used to assess the effect of ambient temperature and the effect of therapy with ICs.

Questionnaires

Parents were asked to complete a modified version of the American Thoracic Society questionnaire on the child's present and past respiratory history [14]. They were also asked to provide detailed information on their child's medication use during the 4 weeks preceding the measurements.

Skin-prick testing

Allergen sensitization was measured by means of skin-prick testing on the volar aspect on both the forearms. Skin prick tests (Soluprick, ALK-Abellö, Horsholm, Denmark) were assessed for common inhaled- and food-allergens in Italy as described elsewhere [4].

Exhaled breath temperature

EBT measurements (in °C) were made with the X-Halo breath thermometer (Delmedica, Singapore, Indonesia). The technique has been fully described by Popov et al. [12,13]. In brief, when a subject exhales through the mouthpiece into a valve, the exhaled air enters a chamber by passing through a copper tube which contains a thermal sensor. Excess air is pushed out of the chamber of the device with every exhalation. The temperature is recorded by the thermal sensor until it stabilizes and equilibrium is reached, usually between 5 and 10 min. As described by the authors, the thermal sensor is calibrated in two points of the range 0–36 °C and the measured value is extrapolated [12]. The manufacturers recommend a confirmation test, bi-weekly or every 100 measurements. It consists in removal of the environmental chamber allowing the device to be exposed to the room environment for a few minutes until the temperature of the device is equal to the room temperature. When the environmental chamber is placed back, an increase in temperature

of the device should be demonstrated by blowing into the device for about 30 s. To control for readings to drift over time, we compared measurements from three different X-Halo breath thermometers. Two of these devices were used for breathing maneuvers in the same session whereas the third device was switched on as a control; readings were compared before each tidal breathing measurement. Measurements were recorded during normal tidal breathing while the subject was in a comfortable sitting position. Measurements were obtained in duplicate, with on an average a 5-min interval, in order to avoid interference due to repeated efforts. Subject body temperature measured with an ear thermometer (Kendall Genius² Tympanic Thermometer, Tyco Healthcare Group LP, Mansfield, MA), ambient temperature and relative-ambient humidity (%RH) measured with a thermo-hygrometer (Lutron HT-305, Lutron Electronic Enterprise CO., LTD, Taipei, Taiwan); temperature range -20 to 60°C , resolution 0.1°C , accuracy -0.8°C , humidity range 10 – 95% RH, resolution 0.1% RH, accuracy $\pm 3\%$ RH) were also recorded.

Fractional exhaled nitric oxide

FE_{NO} was measured with an electrochemical device (HyPair FENO, Medisoftware Group, Zoning de la Voie Cuivrée, Belgium). The single-breath online technique was used as recommended by the ATS/ERS-guidelines [15]. Each subject repeated the exhalation maneuver after a 1–2-min interval; at least two samples were collected from each subject at an expiratory flow of 50 ml/s and expiratory pressure of $10\text{ cm H}_2\text{O}$.

Spirometry

Dynamic volumes and flows were assessed with a portable spirometer (heated Fleisch pneumotachograph ESS- γ , Biomedin s.r.l., Padua, Italy), with subjects in a standing position wearing a nose clip. Duplicate measurements from at least three acceptable maneuvers were obtained [16]. Measurements were expressed either as their absolute values (L or L/s) or as a percent of predicted values [17].

Statistical analysis

The sample size was calculated on the basis of an expected difference of means; $\mu_2 - \mu_1$ in EBT of 1.5°C , when the standard deviation of EBT measurements in a pilot study in a population of asthmatic and healthy subjects using our method was 2.56°C , with a power $(1 - \beta)$ of 80% and a level of significance (α) of 5% , using the following equation: $n = 2(z_{\alpha} + z_{2\beta})^2 \times \sigma^2 / \delta^2$, where $\delta = \mu_2 - \mu_1$. From these calculations, the required number of subjects for each group was 46. After inclusion of all subjects, the expected difference of means in EBT ($\mu_2 - \mu_1$) was recalculated for the actual population of asthmatic and healthy children.

Statistical software (Statistical Package for the Social Sciences (SPSS) for Windows, version 9, SPSS, Inc., Chicago, IL) was used for statistical analysis. The normal distribution of variables was assessed by the Kolmogorov–Smirnov test. The Mann–Whitney test was used to compare two groups. The Wilcoxon signed-ranks test was used to

compare paired data. Degrees of freedom (df) for each comparison were provided. Differences in EBT between groups were adjusted for potential confounders using a multiple linear regression. Pearson's or Spearman correlations were applied according to the normal distribution of variables. The Bland–Altman plot for agreement was used to assess the coefficient of repeatability ($\text{CR} = \text{two standard deviations from the mean difference}$) between two within-session and two within-day EBT measurements [18]. High repeatability, i.e. a low CR value, denotes narrow deviation from the mean difference between two measurements. The intraclass correlation coefficient (ICC) was used to assess reproducibility of measurements. This coefficient estimates the average correlation between all possible ordering of pairs of measurements (two within-session or two within-day EBT measurements). An ICC value of 1.0 denotes perfect reproducibility and a value of 0.0 denotes no different reproducibility than expected by chance [19]. An ICC target of at least 0.6 for repeated measurements was accepted as clinically useful [20]. Statistical significance was defined as p values < 0.05 .

Results

A total of 153 subjects aged 6.2–17.5 years were recruited for this study. Thirteen children from the control group had parent-reported atopy, and 16 children in the patient group had chronic respiratory diseases other than asthma. These children were excluded. From the remaining 124 subjects, 61 subjects (45 males) were enrolled in the patient group and 63 subjects (28 males) were enrolled in the control group. No significant differences in EBT values were found between males and females in the patient group or in the control group (male asthmatic patients vs. female asthmatic patients [32.4 (4.1) vs. 32.8 (1.0), df: 59, $p = 0.207$ by the Mann–Whitney test]; male healthy controls vs. female healthy controls [32.6 (1.8) vs. 33.1 (1.6), df: 61, $p = 0.928$ by the Mann–Whitney test]). Asthmatic children had similar demographic characteristics, lower lung function values, and higher FE_{NO} measurements than healthy children. Fifty-two asthmatic children (85.2%) had at least one positive skin-wheal reaction ($\geq 3\text{ mm}$) to common allergens. Twenty-two patients (36.1%) reported daily use of inhaled Budesonide (200 – 400 mcg per day) during the 4 weeks preceding the measurements. Relative-ambient humidity (%RH) was significantly lower during the measurements in asthmatic children (Table 1).

Reproducibility of EBT measurements

1. Within-session measurements (in duplicate)

Within-session reproducibility in our 124 subjects was high (ICC: 0.95). EBT values tended to increase from the first to the second measurement ($r = 0.88$, $p < 0.001$ by Spearman's rho correlation), suggesting a test–retest situation (Figure 1). The second EBT measurement was significantly higher than the first EBT measurement [33.1 (2.5) vs. 32.5 (2.2), df: 123, $p < 0.001$ by Wilcoxon signed-ranks test]. The mean difference between duplicated EBT measurements was 0.37°C with a CR of 2.05°C ; differences decreased for averaged EBT values above 30°C (Figure 2).

Table 1. Characteristics of study population.

	Asthmatic patients (n = 61)	Healthy controls (n = 63)
Demographics		
Height, cm ^a	145.1 ± 15.7	147.5 ± 12.8
Weight, kg ^a	42.1 ± 14.3	45.7 ± 13.8
BMI, kg/m ^{2a}	19.4 ± 3.3	20.6 ± 4.0
Age, year ^a	11.1 ± 2.8	11.3 ± 2.1
Male/female, n	45/16	28/35
Atopy, n (%)	52 (85.2)	–
FE _{NO} (ppb) ^b	26.5 (27.8) ^c	10.0 (7.0)
EBT (°C) ^b	32.6 (3.3)	33.0 (1.6)
nEBT (°C) ^b	9.4 (3.8) ^d	11.3 (4.2)
Lung function^c		
FVC%	98.8 ± 12.3	101.0 ± 11.2
FEV ₁ %	94.6 ± 13.4 ^c	102.0 ± 9.9
FEV ₁ /FVC%	96.1 ± 9.9 ^c	101.5 ± 6.5
FEF _{25–75} %	81.5 ± 27.9 ^c	103.6 ± 22.3
Therapy with inhaled corticosteroids, n (%)	22 (36.1)	–
Respiratory symptoms last 4 weeks, n (%)		
Dyspnea	21 (34.4)	–
Cough	21 (34.4)	–
Runny nose	17 (27.9)	–
Nasal blockage	29 (47.5)	–
Miscellaneous		
Ear temperature (°C) ^b	36.3 (0.5)	36.3 (0.8)
Relative-ambient humidity (%) ^b	36.0 (26.0) ^c	54.0 (14.0)
Ambient temperature (°C) ^b	22.2 (2.3)	21.3 (1.7)

^aHeight, weight, BMI and age are given as mean ± standard deviation (SD).

^bFractional exhaled nitric oxide, exhaled breath temperature, normalized EBT by ambient temperature, ear temperature, relative-ambient humidity and ambient temperature are given as median and interquartile range (IQR).

^cLung function values are percent of predicted ± SD.

^d*p* < 0.05 asthmatic patients vs. healthy children by the Mann–Whitney test.

^e*p* < 0.005 asthmatic patients vs. healthy children by the Mann–Whitney test.

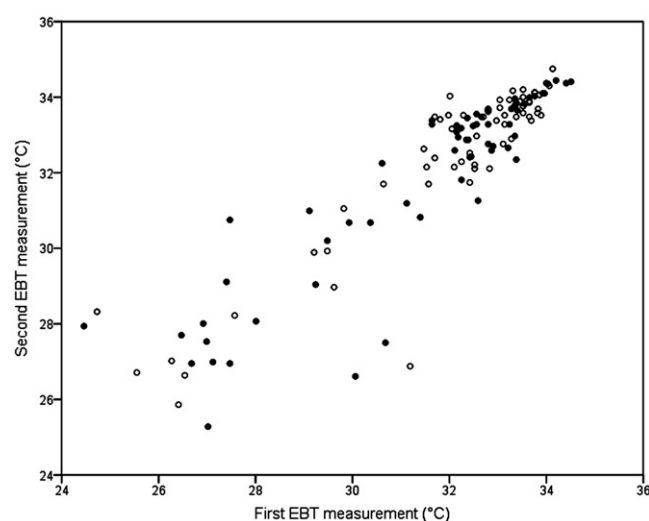


Figure 1. Relationship between the first and second EBT measurement. EBT values tend to increase from the first to the second measurement ($r = 0.88$, $p < 0.001$), suggesting a test–retest situation. The black squares represent asthmatic patients ($n = 61$) and the white squares represent healthy controls ($n = 63$).

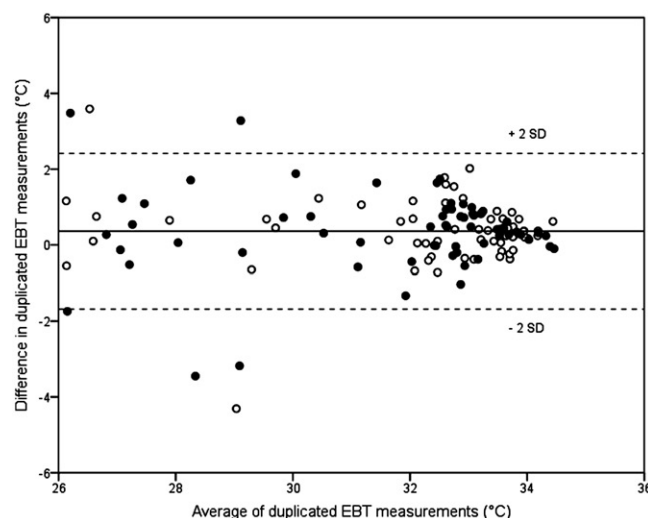


Figure 2. Reproducibility of EBT measurements. The Bland–Altman plot for the difference in duplicated EBT measurements (2nd–1st) and the average of duplicated EBT measurements (1st + 2nd/2). The mean difference between duplicated EBT measurements was 0.37 °C (shown by the horizontal black line) with a CR of 2.05 °C (shown by the two horizontal dotted lines); differences decreased for averaged EBT values above 30 °C. The black squares represent asthmatic patients ($n = 61$) and the white squares represent healthy controls ($n = 63$).

2. Between-session within-day measurements (morning vs. afternoon)

Ten healthy schoolchildren (five males and five females) and three asthmatic patients (two males and one female) performed morning as well as afternoon measurements. The proportion of healthy to asthmatic subjects in this subgroup differed from the total population, we, therefore, described the within-session and between-session reproducibility of this subgroup separately.

No significant difference between median morning and median afternoon EBT measurements was found in the subgroup of 13 subjects [33.2 (1.2) vs. 33.6 (1.6), df: 12, $p = 0.108$ by the Wilcoxon signed-ranks test]. The between-session within-day reproducibility (ICC: 0.78) was slightly lower than the within-session reproducibility (ICC: 0.83) in the same 13 subjects, but it was still high. Similar between-session within-day and within-session mean differences in EBT were found (0.37 °C) with only a small decrease in repeatability for morning–afternoon sessions (between-session CR: 1.41 °C and within-session CR: 1.19 °C).

Effect of measurement conditions on EBT and its normalized value

Both EBT and nEBT correlated with relative-ambient humidity, age, and age-related measurements such as height, weight, and lung function (Table 2). The increasing effect of %RH on EBT among age groups is illustrated by using median values of these variables (age: 11 year, %RH: 46.5%) as cut-off levels (Figure 3) [e.g. among children ≤ 11 year, EBT at %RH < 46.5%: 31.1 (4.7) vs. EBT at %RH > 46.5%: 32.7 (1.1), df: 53, $p = 0.035$ by the Mann–Whitney test].

Table 2. Spearman's Rho correlations with EBT and normalized EBT.

Variable	EBT	nEBT
Age, year	$r=0.47, p<0.001$	$r=0.43, p<0.001$
Weight, kg	$r=0.40, p<0.001$	$r=0.38, p<0.001$
Height, cm	$r=0.41, p<0.001$	$r=0.43, p<0.001$
BMI, kg/m ²	$r=0.26, p=0.004$	$r=0.21, p=0.023$
FVC, l ^a	$r=0.44, p<0.001$	$r=0.42, p<0.001$
FEV ₁ , l ^a	$r=0.45, p<0.001$	$r=0.44, p<0.001$
FEF _{25–75} , l/s ^a	$r=0.35, p<0.001$	$r=0.36, p<0.001$
FE _{NO} , ppb	$r=-0.15, p=0.089$	$r=-0.10, p=0.283$
Ear temperature, °C	$r=-0.01, p=0.955$	$r=-0.07, p=0.472$
Relative-ambient humidity, %	$r=0.23, p=0.017$	$r=0.47, p<0.001$
Ambient temperature, °C	$r=-0.04, p=0.686$	$r=-0.69, p<0.001$ ^b

^aLung function variables are given as absolute values (liters or liters/s).
^bnEBT and ambient temperature are correlated, as expected, because nEBT is obtained by subtracting the ambient temperature from EBT.

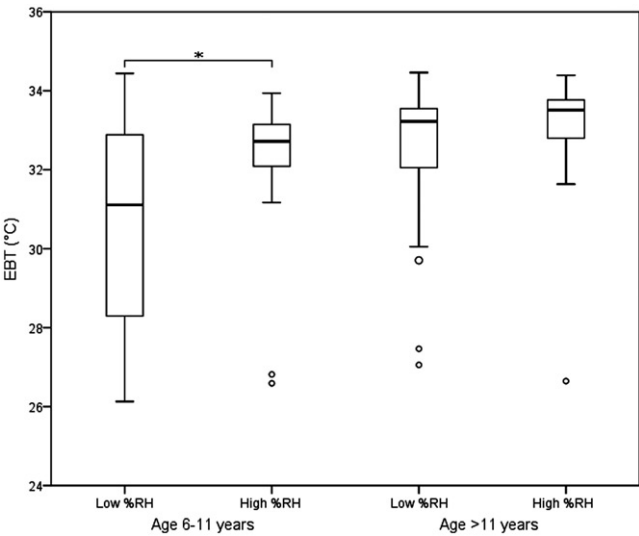


Figure 3. Increasing effect of percent ambient-relative humidity on EBT among two age groups. The median values of percent ambient-relative humidity (%RH) and age were chosen as cut-off levels (%RH: 46.5%, age: 11 year). Percent ambient-relative humidity of $\leq 46.5\%$ represents low %RH, whereas a percent ambient-relative humidity of $>46.5\%$ represents high %RH. Box whisker plots show median, interquartile range, and out-layers (small circles). * $p<0.05$ by the Mann–Whitney test.

Differences in EBT and nEBT between groups

A similar EBT was measured in healthy controls and asthmatic patients [33.0 (1.6) vs. 32.6 (3.3), df: 122, $p=0.080$ by the Mann–Whitney test], even when they were matched by gender. However, higher nEBT was found in healthy controls than in asthmatic patients [11.3 (4.2) vs. 9.4 (3.8), df: 122, $p=0.010$ by the Mann–Whitney test]. All subjects were stratified according to age, because EBT did not differ between healthy and asthmatic children. The median age in our study population was chosen as a cut-off value. EBT in children ≤ 11 years of age was significantly lower than EBT in children >11 years of age [32.4 (4.0) vs. 33.2 (1.2), df: 122, $p<0.001$ by the Mann–Whitney test]. However, a similar EBT was recorded in healthy controls and asthmatic subjects from the same age group [age group ≤ 11 years: 32.6 (3.9) vs. 32.0 (4.6), df: 61,

$p=0.252$ by the Mann–Whitney test and age group >11 years: 33.5 (1.3) vs. 32.9 (1.1), df: 59, $p=0.416$ by the Mann–Whitney test].

Effect of therapy with inhaled corticosteroids

Comparable EBT was found in the 22 asthmatic patients treated with ICs (ICs-treated) and the 39 asthmatic patients who were not treated with ICs (corticosteroid-naïve) [32.5 (4.2) vs. 32.7 (2.9), df: 59, $p=0.674$ by the Mann–Whitney test]. However, nEBT was found significantly lower in ICs-treated asthmatic patients than in those who were corticosteroid-naïve [8.5 (3.9) vs. 10.8 (3.9), df: 59, $p=0.023$ by the Mann–Whitney test]. Similarly, nEBT decreased (ICs-treated vs. corticosteroid-naïve) among asthmatic patients ≥ 11 years [9.6 (4.1) vs. 11.4 (2.0), df: 25, $p=0.003$ by the Mann–Whitney test]. No differences in age nor %RH were found among asthmatic children [ICs-treated vs. corticosteroid-naïve; age (in years): 11.9 ± 3.2 vs. 10.6 ± 2.5 , df: 59, $p=0.085$; %RH: 35.0 (16.0) vs. 41.0 (27.0), df: 59, $p=0.254$, both comparisons by the Mann–Whitney test].

Differences in EBT between groups, adjusting for potential confounders

After inclusion of all subjects, the expected difference of means in EBT ($\mu_2-\mu_1$) was recalculated for the actual population of asthmatic and healthy children. The standard deviation of EBT, after correcting for possible variation due to confounders such as age and %RH, was 1.9°C , with a power ($1-\beta$) of 80%, under a two-sided test with a level of significance (α) of 5%, to detect a difference in mean EBT of 1.0°C when comparing asthmatic patients with healthy children.

The difference in mean (SE) EBT comparing asthmatic children with healthy controls was -0.655 (0.412) $^\circ\text{C}$. However, this crude estimated difference may be confounded by factors related to the ambient conditions of the testing environment (e.g. %RH and ambient temperature) and subject characteristics (e.g. age, sex, BMI or height, and weight) that differed between the asthmatic patients and healthy subjects. When adjusting for ambient conditions using a multiple linear regression, the difference in mean EBT was reduced to -0.066 (0.438) $^\circ\text{C}$. When adjusting for subject characteristics, the difference in mean EBT was in the expected, positive direction: 0.951 (0.821) $^\circ\text{C}$. When adjusted for the set of all potential confounding variables, the difference was even larger: 1.089 (0.786) $^\circ\text{C}$. The adjusted difference of 1.1°C is higher than the expected difference of 1.0°C which was recalculated for the two actually studied groups of asthmatic patients and healthy children.

Discussion

We found that EBT measurements were highly reproducible in our study population, although EBT was influenced by age (and age-related variables) and ambient humidity. EBT values were similar for healthy and asthmatic children; when adjusted for potential confounding variables, EBT values of asthmatic patients exceeded those of healthy children by 1.1°C . In addition, nEBT (the normalized value of EBT by

ambient temperature) did discriminate subjects by the disease group and the current therapy with ICs.

Our study is the first to describe within-session reproducibility of EBT in a large group of healthy and asthmatic children. EBT measurements are still reproducible 6 h apart as compared with within-session (in duplicate) measurements in a small subgroup of children. Reproducibility of EBT with the tidal breathing technique has been previously reported in adults by Popov et al. [12]. In concordance with our findings, the authors described that the reproducibility of EBT measurements, recorded at the same time on subsequent days, in 11 healthy controls, was very high (ICC: 0.99). Reproducibility of EBT with the single breath technique has also been described as satisfactory in three studies [5,6,10]. Taken together, EBT measurements can be considered reproducible; nonetheless, more data are needed to better establish the expected variations with environmental conditions, subject's age, and disease status.

Consistent with the findings of a previous study [21], our study showed that EBT measurements are highly feasible in children. As previously mentioned by Pifferi et al. [22], we also found a learning effect, i.e. the tendency that the second EBT measurement was higher in almost all subjects compared with the first EBT measurement. The difference between the second and the first EBT measurement, attributed to a learning effect, could at least partly reflect inherent variability in the test. More research is also necessary to investigate how much time should elapse between two EBT measurements. Six hours elapsed between the morning and the afternoon sessions without a significant increase in EBT readings in our subgroup of children; however, the small size of this group prevented us to conclude that a potential learning effect disappeared with a prolonged time interval.

The fact that we could not find different EBT values between healthy controls and asthmatic patients is contradictory to previous findings. Popov et al., using the tidal breathing technique in adults, reported significantly higher EBT levels in uncontrolled asthmatic patients than in healthy controls [12]. Other authors, using the single breath technique, found that the rate of EBT increase ($\Delta e^{\circ}T$) [5, 9], or the plateau value at the end of expiration (PLET, $^{\circ}C$) [10,22], distinguished asthmatic subjects from healthy controls. On one hand, with the study of Popov et al. [12] and Xepapadaki et al. [21], we did not include patients with uncontrolled asthma or virus-induced asthma exacerbations; yet our asthmatic children had lower lung function values and higher FE_{NO} levels than their healthy controls, therefore, indicating atopic airway inflammation. Notwithstanding, without a direct physiological measure (e.g. sputum analysis of eosinophilia), we cannot exclude that at least some of our patients may have been in a period of relative health where “actual” airway inflammation was not biologically significantly different to healthy controls. On the other hand, EBT could be less sensitive than FE_{NO} and other exhaled biomarkers in the exhaled breath condensate (EBC) to distinguish asthmatic patients from their healthy controls [23,24]. However, the measurement of biomarkers in EBC for monitoring asthma is impractical and not available in real time as compared with EBT and FE_{NO} .

A possible explanation for the discordance between our results and those previously reported is that EBT measurements were performed at a higher relative-ambient humidity in the school than in our hospital; therefore, EBT values were raised in healthy controls. Indeed, saturated air contains more thermal energy than dry air [25]; this raises the possibility of a mild change in the airway mucosa after multiple breaths in these children. In contrast, a relative-ambient humidity in the range 22–72% did not influence tidal breathing EBT in adults [12]. Nonetheless, it remains difficult to compare our findings with other studies that used a different technique and/or measured EBT in adults only [5,9,10,22].

We found that, in our population, children ≤ 11 years had a lower EBT than children > 11 years of age. However, a similar EBT was measured among healthy controls and asthmatic subjects from the same age group. In two previous studies, no influence of age on EBT was found, probably because only few subjects were analyzed [12,26]. We did find that EBT and nEBT increase with age and %RH. Indeed stratification by age and %RH showed significant differences in EBT and nEBT. This is supported by Kralimarkova et al. [27] who studied EBT with the tidal breathing technique in 78 children. They also found that EBT was influenced by age ($r = 0.43$, $p < 0.001$). We hypothesize that age dependence of EBT can be explained by lung growth, i.e. increasing bronchial/alveolar surface, as suggested by the relationship we found between EBT and absolute values for lung volumes and flows.

Whereas the crude estimated difference in EBT between asthmatic and healthy children was negative, the adjusted difference for potential confounders (ambient conditions and subject characteristics) was positive, i.e. asthmatic patients had close to 1.1 $^{\circ}C$ higher EBT levels than healthy controls. This difference was higher than the expected difference of 1.0 $^{\circ}C$ calculated from the power analysis for the actual size of the groups. Although this finding suggests that EBT measurements actually reflect airway inflammation, it also raises the need of standardization of the test by several potential confounders.

No correlation between EBT and ambient temperature was found. Nonetheless, the potential influence of ambient temperature on EBT could not be excluded, we, therefore, assessed nEBT [6]. nEBT was capable of discriminating subjects by the disease group and the current therapy with ICs. This suggests that, in contrast to previous findings [12], EBT is influenced by ambient temperature [26].

Respiratory heat exchange in the conducting airways increases as the inspired air temperature decreases [28]. Also, elevated respiratory heat and moisture loss have been found to correlate with sputum eosinophil percentage in asthmatic subjects, giving further support to the role for thermal measurements in assessing the increased airway mucosal blood flow associated with airway inflammation [29]. Further studies are necessary to specify in more detail the influence of ambient temperature on EBT when using the tidal breathing technique.

We found that nEBT was lower in the ICs-treated compared with the ICs-naïve asthmatic children. Recent studies in small groups of asthmatic patients reported a decrease in EBT after treatment with corticosteroids [12,30]. These findings suggest that perhaps EBT is more suitable to

monitor inflammatory changes (and may be disease control) in a single patient, instead of distinguishing between healthy and asthmatic subjects. In keeping with this belief, exercise-induced changes in EBT correlate with the degree of exercise-induced bronchoconstriction in asthmatic children [31], adding to the hypothesis that EBT reflects the interaction of several pathological mechanisms of asthma as airway inflammation/remodeling and hyper-reactivity [9,31].

A possible limitation of our study could arise from unequal gender distribution in our study population, with 74% males in the patient group and only 44% in the control group. Gender has been reported to be of no influence on tidal breathing EBT measurements in adult subjects [12]; neither had we found significant differences in EBT between males and females in both our patient and control group. Yet, gender differences in growth of the airways are well documented in children and adolescents [32–34]; how this unequal growth and pubertal status influences EBT measurements awaits further investigation.

Conclusion

Our study demonstrated that measurements of EBT are highly reproducible; however, our results suggest the need of standardization by several potential confounders, including age and ambient humidity. EBT measurements are probably better in monitoring asthma therapy with ICs than to distinguish patients with partially controlled or fully controlled asthma from healthy controls. More studies are needed to improve the usefulness of EBT measured with the tidal breathing technique in the pediatric population.

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Declaration of interest

The authors report no conflicts of interest.

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