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The Evaluation of Changes in the Breath Sound Spectrum with Bronchoconstriction and Bronchodilatation in Asthmatic Children

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Objective: Focusing on the relative-middle sound area of the breath sound spectrum, the relationship between airway changes and breath sounds in asthmatic children was investigated.
Methods: In Study 1, 77 children (6–16 years old) were included. The breath sound parameters, the ratio of the second area to the third area of the power spectrum (A_2/A_3) and the ratio of the third area to the fourth area (B_3/B_4) were evaluated 3 times, before and just after methacholine inhalation and after β_2 agonist inhalation. Other breath sound parameters, the frequency limiting 99% of the power spectrum (F_{99}), the roll-off from 600–1200 Hz (Slope) and the ratio of the third and fourth area to the total area under the curve (A_3/A_T and B_4/A_T), and the ratio of power and frequency at 50% and 75% of the highest frequency of the power spectrum (RPF_{75} and RPF_{50}), were also evaluated. In Study 2, 91 children (6–16 years old) were included, with evaluations performed twice: before and after β_2 agonist inhalation. Spirography and forced oscillation technique were also performed.
Results: In Study 1, A_2/A_3 and B_3/B_4 were significantly increased after methacholine inhalation and decreased after β_2 agonist inhalation ($p < 0.001$, $P < 0.001$, respectively). In Study 2, A_2/A_3 and B_3/B_4 were significantly decreased after β_2 agonist inhalation. These changes in A_3/A_T and B_4/A_T were the inverse of those in other spectrum curve indices.
Conclusions: A_2/A_3 and B_3/B_4, indicate the breath sound changes after bronchoconstriction and bronchodilatation. These parameters may be useful for assessing bronchial reversibility in asthmatic children.

Key words: asthma, breath sound analysis, β_2 agonist, children, methacholine

INTRODUCTION

The diagnosis of asthma in children under five years of age is known to be difficult [1, 2]. However, it has been suggested that breath sounds are sensitive to airway changes [3], and auscultation is useful for physicians because it is a noninvasive and easy-to-perform technique that can be applied to diagnose various respiratory diseases. An objective breath sound analysis is expected to be a safe and simple method for the clinical assessment of airway changes in children [4, 5]. Recent developments in signal processing methods have improved the possibility of extracting clinically relevant information from breath sounds [6].

Even in the absence of audible adventitious sounds, breath sounds show changes when constriction or dilatation of airways is present [7–9]. Parameters are analyzed based on the data of the breath sound spectrum curve of individual patients [5, 6]. Although breath sounds are markedly affected by the maximum airflow rate of the breaths [10, 11], the spectrum curve indices receive only minor influence from the airflow rate because they are reflecting an area ratio and the gradient of the high frequency region to the entire spectrum region [5].

From the clinical viewpoint of asthma exacerbation, the constriction of bronchi causes high-pitched wheezing. It has been reported that the changes in the sound spectrum curve in the high-pitched region are obvious under conditions of bronchoconstriction in children with asthma [12, 13]. In contrast, changes in the relative middle-pitched region of the sound spectrum curve have recently been reported in infants with risk of asthma development [14]. In this report, we focused on the changes in the middle-pitched region of the spectrum curve and retrospectively calculated the ratio of the relative middle-pitched region to the relative high-pitched region after methacholine and β_2 agonist inhalation.

PATIENTS AND METHODS

Study subjects

Seventy-seven pediatric outpatients in Study 1, and 91 patients in Study 2 agreed to participate in this study (Table 1). All of them were treated at Tokai University Hospital from April 1, 2012 to March 31,

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Table 1 Profile of patients
Study 1, Methacholine inhalation test

No.	Age (Years)	Sex (m:f)	FVC (%pred)	FEV_1 (%pred)	FEF_{25-75} (%pred)	Rrs.cont (cmH_2O/L/sec)	Dmin (Unit)	St (cmH_2O/L/sec/min)
77	10*	52:25	80.4	85.0	93.0	7.0	1.8	1.8
	(8, 12)		(73.4, 89.5)	(78.5, 94.3)	(77.9, 108.0)	(5.4, 8.5)	(0.5, 2.8)	(1.0, 2.5)

Study 2, β_2 agonist inhalation test

No.	Age (Years)	Sex (m:f)		FVC (%pred)	FEV_1 (%pred)	FEF_{25-75} (%pred)	R5 [kPa/(L/s)]	R20 [kPa/(L/s)]	X5 [kPa/(L/s)]
91	9	58:33	Before	83.0	92.4	100.6	0.57	0.45	-0.20
	(7, 11)			(76.7, 90.8)	(87.4, 97.4)	(89.0, 116.1)	(0.43, 0.67)	(0.37, 0.55)	(-0.23, -0.15)
			After	84.7	94.0	105.9	0.43	0.39	-0.18
				(76.8, 90.7)	(90.6, 96.7)	(94.2, 127.3)	(0.35, 0.57)	(0.31, 0.47)	(-0.22, -0.14)
			P value	0.763	**0.017**	**< 0.001**	**< 0.001**	**< 0.001**	**0.002**

*; median (first quartile, third quartile), Before; before β_2 agonist inhalation, After; after β_2 agonist inhalation, P value; Wilcoxon's signed-rank test. Bold type indicates a significant difference.

2019. The patients who were treated from April 1, 2012 to July 31, 2015 had participated in previous studies [12, 15]. The inclusion criteria were as follows: ≥ 1 positive specific IgE value (> 0.7 UA/ml), recurrent wheezing and bronchial hyperresponsiveness by methacholine inhalation challenge [16]. All of the participants had been diagnosed with atopic-type asthma by a physician.

Inhaled steroids and leukotriene receptor antagonists were withdrawn for 24 h, and β_2 agonist inhalation was withdrawn for 12 h before the test. Written informed consent was obtained from all of the children or their legal guardians and the study protocol was approved by the institutional review board of Tokai University Hospital (No. 11R-158, approval date; December 21, 2011).

Pulmonary function tests and forced oscillation technique (FOT)

The pulmonary function of the participants was determined via spirometry using a calibrated computerized spirometer (Chestgraph HI-105; Chest Co., Tokyo, Japan). The resting baseline was selected using the best-of-three resting results based on the highest sum of the forced vital capacity (FVC) [17].

The FOT parameters were measured using the impulse oscillometry system (Master-Screen-Impulse Oscillometry System; Jaeger Co., Wurzburg, Germany) [18]. All measurements were made in the standing position with the patient's nose clipped. Real-time recordings of mouth pressure and flow signals pulsed through a 5- to 20-Hz spectrum were superimposed over tidal breathing. Respiratory resistance at 5 and 20 Hz and their difference (R5 and R20, respectively), as well as the respiratory reactance at 5 Hz (X5) were evaluated.

Methacholine inhalation challenge

In Study 1, the methacholine inhalation challenge was performed according to the method described by Takishima [19]. In brief, methacholine was diluted 2-fold with saline on the day of the test to provide a series of 10 strengths ranging from 25 mg/ml to approximately 49 μg/ml [19]. During the methacholine inhalation test, the respiratory resistance (Rrs) was continuously measured using an Astograph® (Chest Co.). Methacholine administration was stopped when the Rrs reached double the baseline value (Rrs.cont) (Appendix 1). The minimum cumulative dose of methacholine to cause bronchial constriction (Dmin), represents the bronchial sensitivity. One Dmin unit was considered equal to the inhalation of 1.0 mg/ml of aerosolized methacholine solution for 1 minute. The speed of bronchoconstriction in response to methacholine (St), which represents the bronchial sensitivity, was also calculated.

β_2-agonist inhalation in Study 2

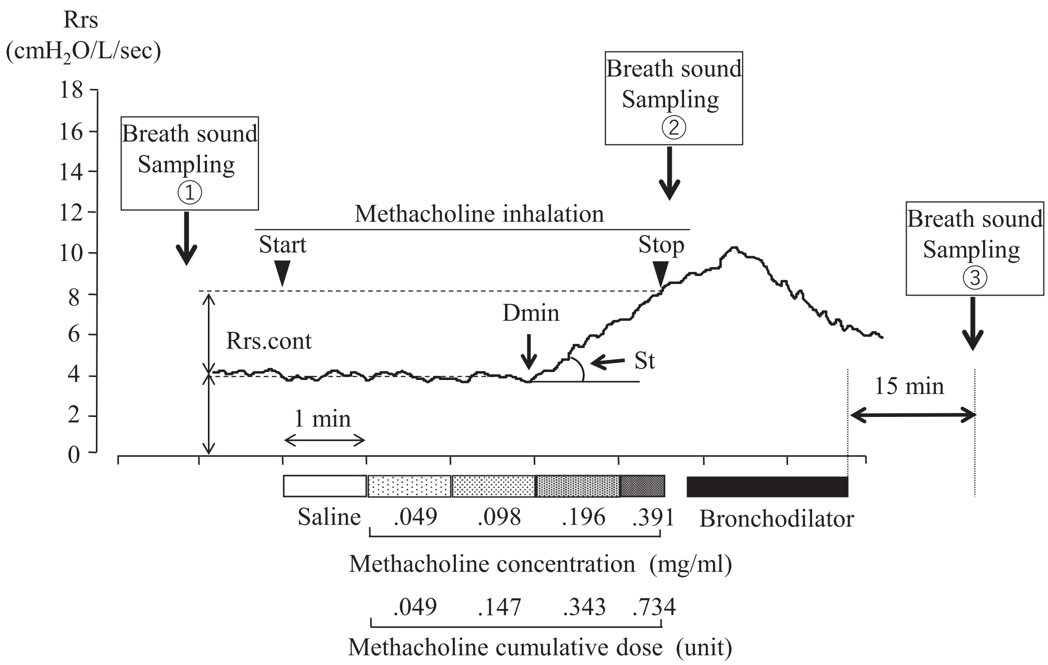
In Study 2, all patients inhaled a β_2-agonist solution (procaterol 30 μg and saline 2.0 ml). Breath sound samples were obtained twice, before and 15 minutes after β_2 agonist inhalation.

Breath sound analyses

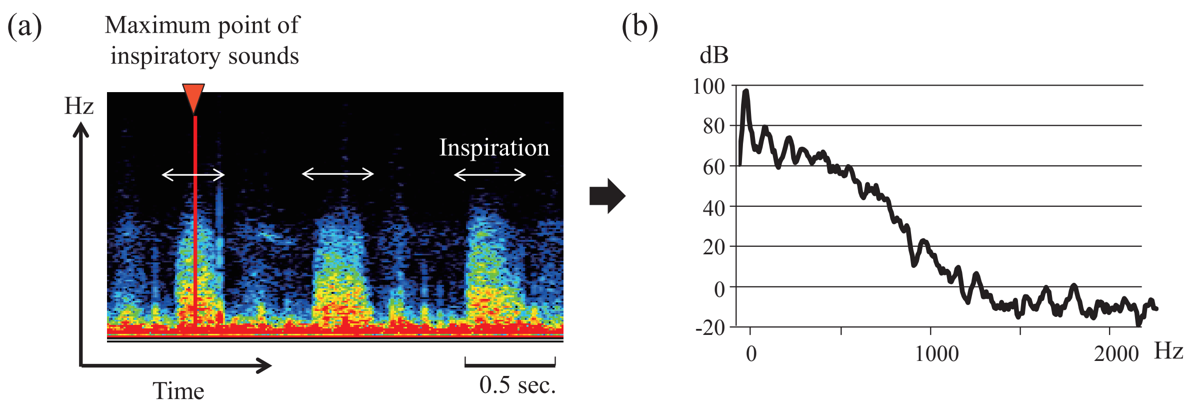
Breath sound analyses were performed for all participants, as described previously [12, 15]. Breath sounds were recorded using a handheld microphone for ≥ 10 seconds in a silent room. The microphone was placed on the right upper anterior chest at the second intercostal space along the mid clavicular line, because that point has the strongest breath sound (mainly bronchial sound) and is the least affected by heart sounds. A sound analysis of the inspiration phase was performed using an LSA-2000 sound spectrometer (Kenz Medico Co., Saitama, Japan).

The sounds were displayed as a spectrograph (Appendix 2). The sound-amplifying unit was found to be effective for analyzing sounds in the range of 100–2500 Hz. The recorded sounds were analyzed according to a fast Fourier transformation. The sampling frequency was 10,240 Hz and the spectra were obtained using a Hanning window. The zero point of the Y axis (0 dB) was decided based on the mean of the background noise, and was considered to be -90 dBm in this report [15].

The point of the maximum frequency (Hz) in the shape (arrowhead) during inspiration was used for the sound spectrum analysis (Appendix 2a) and sound



Appendix 1 Breath sound analyses during the methacholine inhalation challenge
The dose–response curve for the respiratory resistance (Rrs) during the methacholine inhalation challenge using the oscillation method. The Rrs increased with the inhalation of incremental amounts of methacholine. When the Rrs reached exactly twice the baseline value, the administration of methacholine was stopped and a bronchodilator was administered. Three parameters — the Rrs control value (Rrs.cont), the minimum dose of methacholine (Dmin) and the speed of bronchoconstriction to methacholine (St) — were calculated. The breath-sound samples were obtained three times: before methacholine inhalation, just after methacholine inhalation when the Rrs was increased to twice the baseline Rrs value and 15 minutes after $\beta 2$ agonist inhalation.



Appendix 2 Analyses of the sound spectrograph
The spectrograph was displayed after a Fourier analysis, with the vertical axis showing the frequency in Hz and the horizontal axis showing time. (a) The sound intensity of the breath sounds is indicated by the color. The red vertical line indicates the highest frequency of the inspiratory breath sounds. (b) A five-point moving average was used for smoothing to determine the most suitable values of dBm for determining certain checkpoints on the slope for each sound spectrum.

spectrum was constructed (Y axis; dB, X axis; Hz) (Appendix 2b). The data were automatically calculated using a custom software program [15]. The frequency limiting 99% of the power spectrum (F_{99}) was measured as previously described [12, 20–22]. The Slope indicates the roll-off of the middle spectrum curve (-dBm/octave) [22, 23]. The A_T, A_2, A_3, B_3 and B_4 were conventionally calculated by dBm and Hz (1 arbitrary unit [dBm・Hz] on a spectrum image). The spectrum curve indices (RPF_{75} and RPF_{50}) were also calculated [12]. A five-point moving average was used as a smoothing technique to determine the most suitable dBm value for identifying checkpoints in the slope of each sound spectrum. In this study, each personal breath sound parameter was analyzed with a median value from three tidal breaths.

Statistical analyses

The statistical analyses were conducted using the SPSS software program (IBM SPSS Statistics, Version 22 for Windows). The parameters, before methacholine inhalation, just after methacholine inhalation

Table 2 Changes in the parameters of methacholine inhalation test (Study 1)

	① Before methacholine	② Just after methacholine	③15 min after β_2 agonist inhalation	P value ① vs ②	② vs ③	① vs ③
A_T (dBm・Hz)	5830* (4603, 6855)	6300 (5089, 7879)	6064 (4753, 7293)	**0.001**	**0.012**	0.260
F_{99} (Hz)	1010 (832, 1148)	1148 (911, 1426)	1010 (729, 1203)	**< 0.001**	**< 0.001**	0.790
Slope (-dBm/octave)	20.4 (16.4, 28.0)	17.4 (11.2, 25.5)	23.7 (15.7, 30.4)	0.018	**0.011**	0.733
A_3/A_T (%)	12.4 (11.1, 14.2)	10.1 (8.8, 11.6)	12.5 (10.4, 14.5)	**< 0.001**	**< 0.001**	0.911
B_4/A_T (%)	7.8 (6.4, 10.2)	5.9 (4.9, 7.5)	7.9 (6.1, 9.7)	**< 0.001**	**< 0.001**	0.686
RPF_{75} (dBm/Hz)	6.7 (4.8, 8.0)	4.1 (2.9, 5.1)	6.6 (4.9, 8.4)	**< 0.001**	**< 0.001**	0.626
RPF_{50} (dBm/Hz)	5.9 (5.2, 6.7)	4.4 (3.6, 5.2)	6.1 (5.2, 7.1)	**< 0.001**	**< 0.001**	0.181
A_2/A_3 (%)	2.6 (2.2, 3.0)	3.1 (2.7, 3.6)	2.6 (2.3, 3.1)	**< 0.001**	**< 0.001**	0.730
B_3/B_4 (%)	2.7 (2.3, 3.5)	3.3 (2.8, 4.2)	2.7 (2.3, 3.4)	**< 0.001**	**< 0.001**	0.686

n = 77, *; median (first quartile, third quartile), P value; Wilcoxon's signed-rank test. Bold type indicates a significant difference.

and after β_2 agonist inhalation, were compared using Wilcoxon's signed-rank test. Bonferroni's correction was used for comparison among three groups, and the level of statistical significance was set at $p < 0.016$ ($0.05/3 \approx 0.016$). The data are expressed as the median (first quartile, third quartile).

RESULTS

The baseline lung function and the breath sound analyses

All of the subjects successfully underwent spirometry (Table 1). Chest auscultation by a pediatric respirologist revealed no abnormalities in either study.

In each of the subjects, the parameters of the breath sound spectrum were successfully calculated. None of the breath sound images showed wheezing, crackles or distinct outside noises. The shape of the sound spectrum showed good similarity in the same patients.

Changes in the breath sound parameters during methacholine inhalation test in Study 1

An analysis of the data revealed that the spectrum curve indices of the ratio of relative-middle-pitched sound area to the relative-high-pitched sound area in the sound spectrum curve (A_2/A_3 and B_3/B_4) significantly increased after methacholine inhalation and decreased after β_2 agonist inhalation (Table 2, Fig. 1). In contrast, the other spectrum curve indices (A_3/A_T, B_4/A_T, RPF_{50} and RPF_{75}) significantly decreased after methacholine inhalation and increased after β_2 agonist inhalation).

After β_2 agonist inhalation, the values of A_2/A_3 and B_3/B_4 returned to the same as before methacholine inhalation. This tendency was seen in other spectrum curve indices as well.

Changes in the parameters of spirometry and FOT before and after β_2 agonist inhalation in Study 2

In Study 2, the spirometric parameters, FEV_1 and mean forced expiratory flow between 25% and 75% of the FVC (FEF_{25-75}), showed a statistically significant increase after β_2 agonist inhalation (Table 1). Furthermore, all FOT parameters showed statistically significant changes after β_2 agonist inhalation (Table 1).

Changes in the breath sound parameters before and after β_2 agonist inhalation in Study 2

An analysis of the data revealed that the A_2/A_3 and B_3/B_4 significantly decreased after β_2 agonist inhalation (Table 3). In contrast, the other spectrum curve indices (A_3/A_T, B_4/A_T, RPF_{50} and RPF_{75}) significantly increased after β_2 agonist inhalation. The A_T, F_{99} and Slope did not show significant changes.

DISCUSSION

Normal tracheal breath sounds are high pitched with a frequency ranging from 100 to 1,500 Hz and show a drop in power above a cutoff frequency of approximately 800 Hz [23]. Normal bronchial breath sounds include much higher-frequency components than normal breath sounds and exist in the same range as tracheal breath sounds [24]. In this report, we analyzed stable inspiratory breath sounds that mainly consisted of tracheal and bronchial breath sounds [5].

Based on the frequency, one of the more reliable classifications of normal respiratory sounds is as follows: low (under 100 Hz), middle (200–600 Hz) and high frequency (600–1,200 Hz) [25]. Previous trials in asthmatic children mainly focused on the increase in high-pitched breath sound with bronchoconstriction [5, 15, 26]. It is clinically convincing that children with asthma show an increase in the high-pitched area of

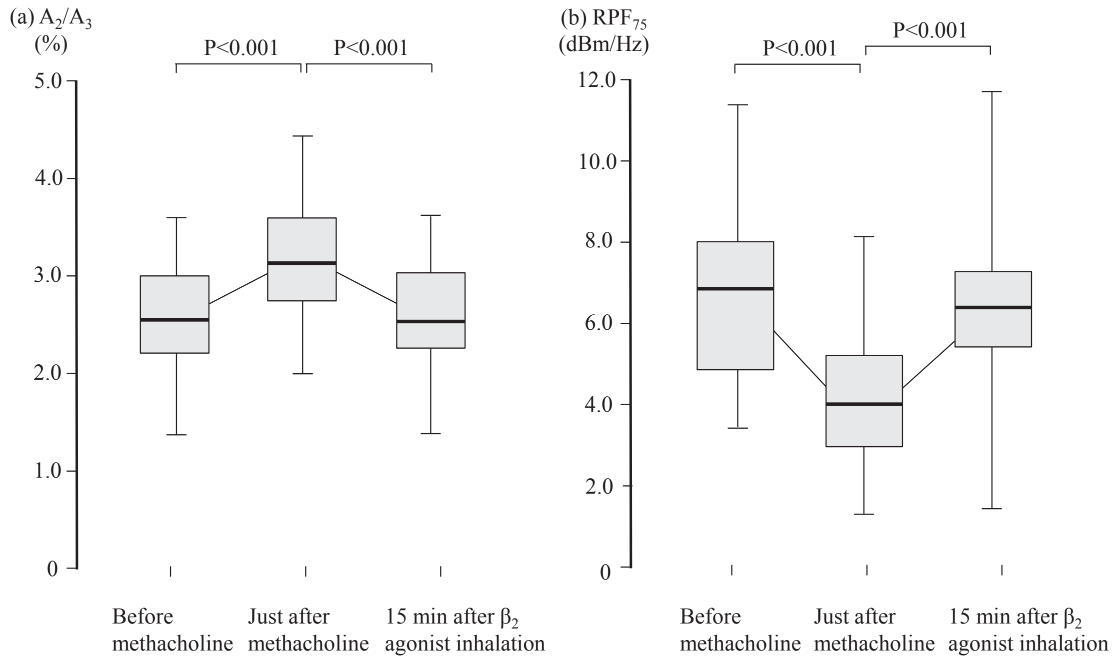


Fig. 1 Changes in breath sound parameters
The A_2/A_3 statistically increased after methacholine inhalation and decreased after β_2 agonist inhalation. In contrast, the RPF_{75} significantly decreased after methacholine inhalation and increased after β_2 agonist inhalation.

>1,000 Hz [13]. However, the middle-pitched breath sounds in asthmatic children have not been studied in details.

In the present study, we focused on the relative middle-pitched breath sounds, and based on the characteristics of the spectrum curve indices used in previous analyses [14], we used the ratio of the middle-pitched area to the high-pitched area of the sound spectrum for our evaluation. The relative middle-pitched sound in this report refers to approximately 340–590 Hz in A_2 and 460–640 Hz in B_3, as the median of F_{99}. F_{99}, which is 99% of the highest frequency of the sound spectrum and differs among individuals, was 940 Hz in the total subjects (n = 168) of this study.

In Study 1, the A_2/A_3 and B_3/B_4 values were significantly increased after methacholine inhalation and decreased after β_2 agonist inhalation. Furthermore, in Study 2, the A_2/A_3 and B_3/B_4 values were also decreased after β_2 agonist inhalation. These changes in the A_2/A_3 and B_3/B_4 values were the inverse of those in the A_3/A_T and B_4/A_T values. The A_3/A_T and B_4/A_T values are the ratio of the higher frequency areas (A_3 and B_4) to the total area (A_T) of the sound spectrum. The decrease in the A_3/A_T and B_4/A_T values after methacholine inhalation resulted in an increase in the high-pitched area, resulting in a prolonged right end of the sound spectrum triangle [15]. In contrast, A_2/A_3 and B_3/B_4 are the ratio of the relative-middle frequency areas (A_2 and B_3) to the relatively higher-frequency areas (A_3 and B_4) of the sound spectrum. Since the increase in the A_2/A_3 and B_3/B_4 values and the decrease in the A_3/A_T and B_4/A_T values may be induced by the same reaction in the sound spectrum curve, we were able to recognize dynamic changes in the sound spectrum curve with bronchoconstriction and bronchodilatation based on the clear changes in these parameters.

Our comparison of the sensitivity in the sound spectrum curve indices showed that the changes in the A_2/A_3 and B_3/B_4 values were more sensitive to airway changes than the changes in the common parameters of F_{99} and Slope. Pediatricians need an objective method of assessing the real time-airway condition. We believe that the sound spectrum curve indices are useful biomarkers for children, as this method will enable the evaluation of the airway condition during tidal breathing within just 30 seconds [5, 15]. A_2/A_3 and B_3/B_4 can be effectively used as clinical biomarkers of airway narrowing in children. Furthermore, in Study 1, our finding that the values of A_2/A_3 and B_3/B_4 after β_2 agonist inhalation returned to the same as those before methacholine inhalation (Table 2) demonstrates the reliability of these parameters. However, the rate of change in the A_2/A_3 and B_3/B_4 values did not show a relationship with the rate of changes in the FEV_1 (data not shown). We also compared the usefulness of A_2/A_T and B_3/A_T; however no remarkable trend was seen under conditions of bronchoconstriction or bronchodilatation (data not shown).

Since breath sounds are affected by the maximum airflow rate of breaths [10, 11] and body height [27], performing an evaluation with the absolute power of the breath sounds in children is difficult. However, as described in previous reports [12, 15], the high-pitched sound is considered to be incorporated into the normal breath sound under conditions of bronchoconstriction and subtracted under conditions of bronchodilatation. It is therefore conceivable that the increase in the high-pitched area, which extended to the right direction of the spectrum curve, induced a decrease in the rate of the relative high-pitched area.

However, there is a possibility that the middle-pitched sound directly increased during bronchoconstriction. It has been reported that infants with atopy showed a direct increase in the middle-pitched

Table 3 Changes in the breath sound parameters before and after β_2 agonist inhalation (Study 2)

	Before β_2 agonist inhalation	15 min after β_2 agonist inhalation	P value
A_T (dBm・Hz)	8905 (8340, 10338)	9209 (7289, 9801)	0.098
F_{99} (Hz)	885 (780, 1040)	890 (760, 1050)	0.936
Slope (-dBm/octave)	23.9 (17.6, 30.8)	25.0 (19.7, 33.2)	0.574
A_3/A_T (%)	11.7 (10.7, 13.8)	13.0 (12.1, 14.5)	**< 0.001**
B_4/A_T (%)	6.9 (5.8, 7.9)	7.7 (7.1, 8.8)	**< 0.001**
RPF_{75} (dBm/Hz)	5.5 (4.6, 7.0)	7.1 (6.2, 8.4)	**< 0.001**
RPF_{50} (dBm/Hz)	5.8 (5.3, 6.6)	7.0 (6.3, 7.8)	**< 0.001**
A_2/A_3 (%)	2.9 (2.6, 3.3)	2.6 (2.4, 2.9)	**< 0.001**
B_3/B_4 (%)	2.8 (2.5, 3.3)	2.5 (2.3, 2.9)	**< 0.001**

n = 91, *; median (first quartile, third quartile), P value; Wilcoxon's signed-rank test. Bold type indicates a significant difference.

part of the spectrum curve indices [14], and some obstruction or dysfunction of the airways may induce an additional middle-pitched part of the spectrum curve indices. In addition, the breath sound of rattles shows a middle-pitched sound (< 600Hz) [28]. We believe that the parameters related to middle-pitched sounds are useful for the multilateral analysis of airway changes in asthmatic children, and intended to explore the optimal use of each breath sound parameters for evaluating lung function from multiple perspectives in all wheezing diseases in children.

Regarding limitations associated with our study, we were unable to suggest directly which part of the airway caused the observed breath sounds. Inoue *et al*. showed that adult asthma was mainly affected by the central respiratory tract according to computed tomography evaluations [29]. In contrast, patients with tracheal stenosis demonstrate an increase in the peak spectral power at 1,000 Hz with an increase in the mean spectral power from 600 to 1,300 Hz [30]. To identify the portion of the airway responsible for the additional sound generation, we will explore this issue in future studies.

A breath sound analysis is an objective approach for assessing the lung function in children. In the present study, we observed dynamic changes in the breath sounds related to airway narrowing, and suggested that fine changes in the airway may be able to be assessed using the sound spectrum curve indices, A_2/A_3 and B_3/B_4. Given the results of Study 1 and Study 2, these parameters may be useful for detecting reversible airway narrowing in infants and younger children from a new angle.

CONFLICT OF INTEREST

The Environmental Restoration and Conservation Agency of Japan in fiscal years 2009-2014

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DISCLOSURE

This study was supported by the Environmental Restoration and Conservation Agency of Japan in 2009-2014.

ABBREVIATIONS

FVC: forced vital capacity
FEV_1: forced expiratory flow and volume in 1 second
FEF_{25-75}: mean forced expiratory flow between 25% and 75% of the FVC
FOT: forced oscillation technique
Rrs: respiratory resistance
Rrs.cont: Rrs control value
Dmin: minimal dose of methacholine
St: speed of bronchoconstriction to methacholine
R5: resistance at 5 Hz
R20: resistance at 20 Hz
X5: reactance at 5 Hz
HFp: highest frequency of the mV2 power spectrum
HFz: highest frequency of the dB power spectrum
AUC: area under the curve
Slope: roll-off from 600 to 1200 Hz
P_T: total power area of 100 Hz to the highest frequency of the power spectrum
A_T: total area under the curve of 100 Hz to the highest frequency of the power spectrum
A_2: the second area under the curve
A_3: the third area under the curve
B_3: the third area under the curve
B_4: the forth area under the curve
F_{99}: frequency limiting 99% of the power spectrum
RPF_{50}: ratio of power and frequency at 50% of the highest frequency of the power spectrum

RPF_{75}: ratio of power and frequency at 75% of the highest frequency of the power spectrum

AUTHORS' CONTRIBUTIONS

Drs. SM and HM designed the study. Drs. HT and ME collected the data. Drs. HF and FN analyzed the data. The final manuscript has been read and approved by all of the authors.

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