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Fractional exhaled nitric oxide in Chinese children with asthma and allergies—A two-city study

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Summary
Fractional exhaled nitric oxide (FeNO) is a non-invasive biomarker of eosinophilic airway inflammation. Our aim was to study associations between FeNO in Chinese children in two cities and asthma, asthmatic symptoms, rhinitis, eczema, and selected childhood and home environmental factors.

A random sample of children in Shanghai ($n = 187$) and Taiyuan ($n = 127$), and additional randomly selected children reporting current wheeze ($n = 115$) were invited for FeNO measurements by NIOX MINO. A questionnaire survey was performed among all subjects (12–14 y) in 59 classes in Shanghai and 44 in Taiyuan. Associations were studied using multiple linear regression using 10log transformed FeNO data and mutual adjustment.

The geometric mean FeNO in the random sample (GM ± GSD) was higher in Shanghai (16.2 ± 1.9 ppb) as compared to Taiyuan (12.8 ± 1.6 ppb) ($P < 0.001$). In the total material ($n = 429$), Shanghai residency ($P = 0.001$), male gender ($P = 0.02$), parental asthma/allergy ($P = 0.04$), doctors' diagnosed asthma (DDA) ($P < 0.001$) and current wheeze ($P < 0.001$) were associated with higher FeNO levels. In non-wheezers ($n = 291$), Shanghai residency ($P = 0.007$), male gender ($P = 0.002$), DDA ($P = 0.04$), current rhinitis ($P = 0.004$) and

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reported pollen/furry pet allergy ($P = 0.04$) were positively associated with FeNO. In wheezers ($n = 138$), DDA was the only significant factor ($P = 0.009$). In conclusion, male gender, current wheeze, DDA, parental asthma/allergy, current rhinitis, pollen/furry pet allergy can be independent determinants of increased FeNO. The lower level of FeNO in Taiyuan is in agreement with previous studies showing lower prevalence of asthma and allergy in Taiyuan as compared to Shanghai.

Introduction

There is a concern about the global increase of asthma and allergies, especially in children. A repeat multi-country cross-sectional survey within the International Study on Asthma and Allergies in Childhood (ISAAC) showed an increase of asthma in middle-income countries especially in Asia.[1,2] A national multi-centre study has demonstrated an increase of physician diagnosed asthma in China during 1990–2000. Shanghai had the highest prevalence of doctors' diagnosed asthma (3.34%) among all cities in 2000.[3]

Fractional exhaled nitrogen oxide (FeNO) is a non-invasive surrogate marker of airway inflammation[4-6] and chronic airway inflammation is a key characteristic in the pathogenesis of asthma.[7] Allergic rhinitis has been reported to be associated with elevated levels of FeNO.[8] Moreover, subjects with a combination of asthma and atopic dermatitis may have higher levels than those with asthma only.[9] The level of FeNO can be influenced by personal and environmental factors. The main personal factors include age, gender, race, height, nitrate intake and tobacco smoking[4,10–12] and environmental factors include both outdoor and indoor air pollution.[13–17] Males were reported to have higher FeNO levels than females,[4] and Chinese schoolchildren had higher FeNO levels than Caucasian ones.[12] There is a high prevalence of home environmental tobacco smoking (ETS) among Chinese children.[18] For normal healthy children, one study reported that the average FeNO level was 11.22 ppb (Geometric average, 95% CI 4.17–30.20) in Chinese children aged 11–14 y.[19] Heavy outdoor air pollution might induce higher FeNO levels (13–16), and one Chinese study reported that FeNO in school children was decreased during the 2008 Beijing Olympics, when air pollution concentrations were reduced.[20]

We have previously published studies in junior high schools in two Chinese cities (Shanghai and Taiyuan) combining measurements of air pollution inside and outside the schools with questionnaire data on asthma, respiratory symptoms and allergies in the pupils.[18,21] Taiyuan (4 million inhabitants) is the largest city in Shanxi province and has very high levels of air pollution, especially in the winter, due to coal burning. Shanghai is a costal well-developed mega-city (20 million inhabitants) with traffic exhausts as the major pollution source. Despite much higher levels of particle pollutants (PM_{10}) and SO_2 in Taiyuan as compared to Shanghai, we found that the prevalence of doctors' diagnosed asthma and pollen or furry pet allergies was much lower in Taiyuan as compared to Shanghai. Because of this observation, we decided to perform a study use FeNO as an indicator of respiratory health in school children in the two cities.

We invited random sample of schoolchildren in Taiyuan and Shanghai and additional randomly selected children reporting current wheeze for FeNO measurements. Our hypothesis was that FeNO levels were associated with asthma, asthmatic symptoms and reported allergic diseases including allergic rhinitis and eczema. Moreover we investigated associations between FeNO and personal factors (gender, BMI, tobacco smoking) and proxy variables for indoor air pollution in the dwelling (ETS, dampness or molds at home), childhood microbial stimulation (rural vs. urban childhood) and current outdoor air pollution situation (Taiyuan vs. Shanghai).

Materials and methods

Study population

This study is based on two previous cross-sectional questionnaire studies on asthma and allergic diseases in Shanghai ($N = 1930$; mean age 12.6 y) and Taiyuan ($N = 2134$; mean age 13.7 y) performed in winter season 2009. Ten randomly selected junior high schools in the urban area of the cities included (totally 59 classes in Shanghai and 44 classes in Taiyuan). School headmasters were contacted and all agreed to participate in the study.

For this clinical study, participants in the previous cross-sectional studies were randomly selected in both cities (Step 1) (Fig. 1). In Shanghai, 187 were recruited including 17 children reporting current wheeze (last 12 months). In Taiyuan, totally 127 were recruited including 6 with current wheeze. Wheezing subjects refer to pupils with confirmative answers "yes" to "Have you ever had wheezing or whistling in the chest in the past 12 months" and non-wheezing subjects with the answer "No". Secondly, additional children with current wheeze were added by randomly selection of wheezers from all wheezing children not included in the random samples (Step 2). In Shanghai, 83 out of 169 additional wheezers were finally recruited and in Taiyuan, 32 out of 69 were finally recruited. The combined group including the random sample as well as the additional wheezers was called "enriched sample". The total enriched sample ($N = 429$) consisted of 138 wheezing subjects (100 from Shanghai and 38 from Taiyuan) and 291 non-wheezing subjects (170 from Shanghai and 121 from Taiyuan). In the whole selection process (Fig. 1.), if there were subjects who did not agree to participate or had any type of current asthma medication, or could not complete the FeNO test, they were excluded in different steps. Informed written consent was obtained from children's parents or legal guardians. The study was approved by the ethical committee of the School of Public Health, Fudan University, Shanghai, P. R. China.

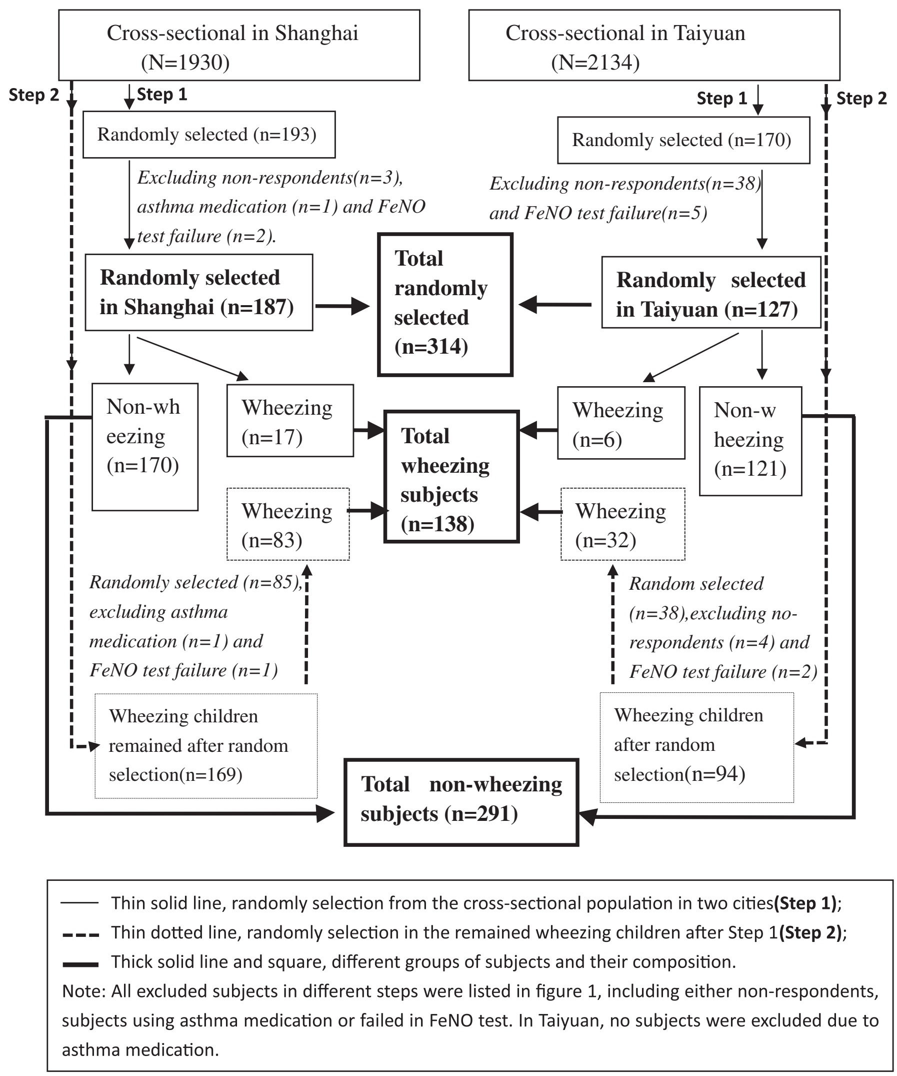

Figure 1 Subjects randomly selected and by wheezing and non-wheezing enriched in Shanghai and Taiyuan, China.

Air pollution measurements

Outdoor levels of NO_2, SO_2 and O_3 was measured outside the schools during the study period at one representative outdoor location per school by diffusion sampling. The same methodology was used in the previous school studies in Shanghai[21] and Taiyuan.[18] Diffusion samplers from IVL Swedish Environmental Research Institute Ltd., Gothenburg, Sweden, were used (www.ivl.se). The sampling time was 7 days. The samplers were placed 2.5—3.5 m above the ground, protected from rain and snowfall in a special plastic box. Totally 10 schools in Shanghai and 10 schools in Taiyuan had outdoor measurements. The concentrations were calculated by the IVL-laboratory and were reported as mean values across the 7-day measurement period.

Assessment of medical data

We used the same questionnaire as in the previous school environment studies. It contained questions on personal and demographic data including age, height, weight, as well as questions on early life factors, current home environment and diet. The questionnaire was distributed in all selected classes and collected back by trained school staff. It was brought home by children and answered together with children's parents or legal guardians, who gave written consent. The FeNO measurements were performed the same month as the questionnaire survey in winter season.

Information on children's health, collected by questionnaire, included doctors' diagnosed asthma, different allergies, current asthma and respiratory symptoms. The questions were adapted from the ISAAC study,[1] the European Respiratory Health Survey (ECRHS),[22] and previous school studies.[18,21,22] This publication was focusing on questions on asthma and asthmatic symptoms included in the ECRHS study, while the questions on current rhinitis and current dermatitis were from the ISAAC study. The question on cumulative prevalence of asthma and current wheeze was identical in ISAAC and ECRHS. There was one set of questions asking about asthma, including doctors' diagnosed asthma (DDA) and current asthma medication. Another set of questions asked about airway symptoms related to asthma during the last 12 months, without using the phrase "asthma". These symptoms included: 1) wheezing or whistling in the chest; 2) at least one daytime attack of shortness of breath during exercise or at rest; 3) at least one night-time awakening with attacks of breathlessness or tightness in the chest and dry cough at night. Moreover, the questionnaire asked about smoking habits, allergy to cat, dog and pollen, and parental allergy/asthma or eczema. For example, the question on cat allergy was: Do you have allergy to cats (yes, no, do not know)? The answer "Not know" and "No" was coded 0, "Yes" was coded 1. There were no questions on parental social economic status.

Questions on early childhood exposure and current dwelling

There was one question asking if the child had been growing up in a city or in the countryside during the first 2 years of life, and another question on domestic exposure to environmental tobacco smoke (ETS) the first year of life. In addition, the questionnaire contained questions on exposure indicators in the current home environment, including ETS at home (yes/no), current building dampness and indoor mould growth the last 12 months.

FeNO test

The FeNO measurement was performed by the portable NIOX MINO® machine (Aerocrine AB, Solna, Sweden). Briefly, pupils were required to sit, rinse the mouth by drinkable pure water first, and then empty theirs lungs by complete expiration. Then they inhaled the NO-free air through the disposable filter to the lung capacity. Finally, they exhaled the air through the machine at an exhalation rate of 50±5 ml/s. One single measurement was collected for each participant. The standard mode, using 10 s exhalation time, was used in all tests. Maximum 5 repeated tests with 10 min rest between were allowed for children who could not complete the test successfully the first time. No nose clip was used. The sensor in the device was replaced periodically according to the manufacture's guide.

Statistical analyses

The FeNO values had an approximately log-normal distribution, thus geometric mean and geometric standard deviation (GM ± GSD) were reported. Independent samples *T* test was applied to study differences in log-transformed FeNO levels between groups. Multiple linear regression analysis was applied to evaluate associations between the investigated factors (demographic, medical, and environmental) and FeNO values (log-transformed data). The factors included center, gender, overweight or obesity (BMI), parental asthma/allergy or eczema, doctors' diagnosed asthma, current wheeze, daytime attacks of breathlessness, dry cough at night, current rhinitis and pollen or furry pet allergy. All factors were included in the models (mutual adjustment). Overweight and obesity was categorized according to the updated BMI reference norm for Chinese children and adolescents.[23] In addition, multiple logistic regression analysis was applied to evaluate the associations between FeNO as an independent variable and six different medical outcomes, adjusting for center, gender, BMI and parental asthma/allergy or eczema. Two sided tests and a 5% significance level was applied, using SPSS 11.5 statistical package.

Results

Air pollution levels

The air pollutants levels of SO_2, NO_2 and O_3(Mean ± SD) measured outside the schools during the study period in Shanghai and Taiyuan were: SO_2 39 ± 4 $\mu g/m^3$ vs. 182 ± 51 $\mu g/m^3$, NO_2 72 ± 10 $\mu g/m^3$ vs. 47 ± 4 $\mu g/m^3$, O_3 20 ± 13 $\mu g/m^3$ vs.18 ± 5 $\mu g/m^3$ (All $p < 0.001$). All data were 7 days average values.

Prevalence of asthma and related symptoms in the random and enriched samples

In the random samples, doctors' diagnosed asthma (DDA) was reported by 7.0% (22/314) with 3.9% (5/122) and 9.1% (17/187), respectively in Taiyuan and Shanghai. Current rhinitis was reported by 34.4% and cat,dog or pollen allergy by 5.7% of the subjects. In wheezers, DDA was reported by 32.0% and in no-wheezers by 4.0% ($p < 0.001$)(data not shown). For different symptoms in wheezers and non-wheezers, 45.7% vs. 12.0% had dry cough at night, 67.4% vs.16.5% daytime attacks of breathlessness, 14.5% vs. 2.1% nighttime attacks of breathlessness, 74.6% vs.30.6% current rhinitis, 17.4% vs.2.4% current eczema and 22.5% vs. 5.2% reported pollen or furry pet allergy. All these differences were highly significant ($p < 0.001$) (data not shown).

FeNO levels, personal factors, early childhood and home environment

In the random sample with the mean age of 13 y, half were boys, and very few were tobacco smokers (Table 1). The majority (>75%) had been exposed to environmental tobacco smoke (ETS) at home, both during early childhood (<1 y) and in the current dwelling. Moulds and signs of dampness or water leakage in the current dwelling were relatively common (8.9–26.0%). About 30% of the children had been growing up in the country side before they were 2 years old. When comparing demographic data and environmental exposure between wheezers and non-wheezers, wheezers had higher FeNO levels, were more often boys, had higher body mass index (BMI), and had more often parents with asthma/allergy or dermatitis (heredity). Moreover, wheezers had more often dampness and indoor mould growth in the current dwelling as compared to non-wheezers.

Initially we compared FeNO levels in the random samples from the two cities (Table 2). The level of FeNO was higher in Shanghai as compared to Taiyuan. Boys had significantly higher level of FeNO than girls in Taiyuan. Those with heredity had significantly higher levels of FeNO in Shanghai. Wheeze, physician diagnosed asthma and daytime attacks of breathlessness were associated with

Table 1 Demographics, home environment and FeNO values in randomly selected and enriched wheezing and non-wheezing children.

	Randomly selected[a]			Wheezing/non-wheezing enriched(n = 429)[a]	
	Total (n = 314)	Shanghai (n = 187)	Taiyuan (n = 127)	Wheezing (n = 138)	Non-wheezing (n = 291)
Age (mean ± SD)	13.0 ± 1.0	12.6 ± 0.8	13.7 ± 1.1***	12.9 ± 0.9	13.0 ± 1.0
Boy (%)	49.4	47.1	52.8	60.9*	50.2
Height (cm)	160.0 ± 8.3	160.1 ± 7.3	159.5 ± 9.7	160.5 ± 8.1	159.5 ± 8.4
Weight (kg)	48.4 ± 10.5	48.6 ± 9.9	48.1 ± 11.5	50.5 ± 10.8	48.0 ± 10.3
BMI (mean ± SD)	18.8 ± 3.3	18.9 ± 3.1	18.8 ± 3.5	19.5 ± 3.2*	18.8 ± 3.2
Overweight and obesity(%)[b]	14.0	15.0	12.6	18.1	13.4
Parental asthma, allergy or eczema (%)[c]	14.0	18.2	7.9*	31.2***	12.0
Own smoking (%)	0.3	0.5	0	1.5	0.4
Home ETS before 1 y age (%)	78.7	80.7	75.6	71.7	67.4
Current home ETS exposure (%)	76.5	74.9	78.9	76.7	76.7
Water leakage or home dampness the last 12 months (%)	22.9	22.0	26.0	35.3**	21.8
Mold at home (wall/ceiling/floor) the last 12 months (%)	11.5	13.7	8.9	20.0**	9.9
Lived in urban areas before 2 y age (%)	70.4	66.1	80.6**	78.2	71.1
FeNO (GM ± GSD, ppb)	14.8 ± 1.8	16.2 ± 1.9	12.8 ± 1.6***	24.8 ± 2.1***	13.8 ± 1.7

GM = geometric mean; GSD = geometric standard deviation; home ETS exposure = environmental tobacco smoke at home.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[a] Comparisons were made between randomly selected subjects in Taiyuan and Shanghai, and between wheezing and non-wheezing subjects.
[b] Overweight and obesity were categorized according to the BMI reference norms for Chinese children and adolescents.
[c] Parental asthma, allergy or eczema refers to any positive answer on father or mother's physician-diagnosed asthma, allergy or eczema.

higher FeNO levels in both cities, while dry cough at night was associated with FeNO only in Shanghai. Current rhinitis and pollen or pet allergy were associated with FeNO in Shanghai. When analyzing the same associations in the enriched samples from the two cities, all factors significantly associated with FeNO in any or both of the cities, were significantly associated in the total material. No associations were found between FeNO levels and any early childhood factors or exposures in the current dwelling.

As a next step we analyzed associations for FeNO, stratified by wheeze (Table 3). In wheezers, FeNO levels were higher in Shanghai as compared to Taiyuan, and higher in those with parental asthma/allergy, doctors' diagnosed asthma, cat allergy and dog allergy. In subjects without current wheeze, more factors were associated with FeNO, including Shanghai residency, male gender, high BMI, parental asthma/allergy, doctors' diagnosed asthma, current rhinitis and pollen allergy.

Then we performed multivariate analysis by multiple linear regression, using 10-log transformed FeNO data as independent variable, and mutual adjustment (Table 4). Four different models were used. In the combined random samples from the two cities, Shanghai residency, male gender, doctors' diagnosed asthma, current wheeze, current rhinitis and pollen or furry pet allergy were independently associated with FeNO. In the combined enriched sample, Shanghai residency, male gender and parental asthma/allergy were significant factors. Then we stratified data with respect to wheeze. Among wheezers, doctors' diagnosed asthma was the only factor significantly associated with FeNO. Among non-wheezers, Shanghai residency, male gender, doctors' diagnosed asthma, current rhinitis and pollen or furry pet allergy were independently associated with FeNO.

Finally we used multiple logistic regression analysis, to analyze associations between FeNO as an independent variable and six health variables, adjusting for centre (Shanghai vs. Taiyuan), gender, BMI, and parental asthma/allergy (Table 5). Five different models were used. FeNO was positively associated with doctors' diagnosed asthma, current wheeze, daytime breathlessness, dry cough at night, current rhinitis and pollen or furry pet allergy, both in the random sample and the enriched sample (a borderline significance of dry cough in the enriched sample). In non-wheezers, FeNO was still associated with doctors' diagnosed asthma, daytime breathlessness, current rhinitis and pollen or furry pet allergy. In those without rhinitis, FeNO was associated with doctors' diagnosed asthma, wheeze and daytime attacks of breathlessness. Finally, in those without pollen or furry pet allergy, FeNO was still associated with doctors' diagnosed asthma, daytime breathlessness, dry cough at night and current rhinitis.

Normal range of FeNO values

In order to determine the normal range of FeNO in healthy Chinese children, GM with a 95% CI for individual values was

Table 2 Associations between FeNO levels (GM ± GSD)(*n*) And selected risk factors in the random sample, in Shanghai ($n = 187$), in Taiyuan ($n = 127$) and in the total material of wheezing/non-wheezing children ($n = 429$).

	Randomly selected sample ($n = 314$)				Wheezing and non-wheezing enriched sample ($n = 429$) [a]	
	Shanghai ($n = 187$) [a]		Taiyuan ($n = 127$) [a]			
	Yes	No	Yes	No	Yes	No
Risk factors						
Center (Shanghai = yes)[b]	16.2 ± 1.9***	12.8 ± 1.6	18.5 ± 2.0(270)***	13.9 ± 1.7(159)		
Boy	17.7 ± 1.8(88)	15.1 ± 1.9(99)	13.9 ± 1.5(67)*	11.7 ± 1.6(60)	18.2 ± 1.9(230)**	15.0 ± 1.9(199)
Overweight and obesity	16.4 ± 1.8(28)	16.2 ± 1.9(159)	14.7 ± 1.5(16)	12.6 ± 1.6(111)	18.5 ± 1.8(64)	16.4 ± 2.0(365)
Parental asthma/allergy/eczema	24.0 ± 2.2(34)***	14.9 ± 1.7(153)	12.9 ± 2.1(10)	12.8 ± 1.5(117)	23.6 ± 2.3(78)***	15.4 ± 1.8(351)
Current Home ETS exposure	16.5 ± 1.9(134)	15.7 ± 2.0(53)	12.9 ± 1.6(97)	12.7 ± 1.5(30)	16.5 ± 1.9(316)	17.0 ± 2.0(113)
Water leakage/home dampness	17.3 ± 2.0(40)	15.8 ± 1.8(137)	12.5 ± 1.5(32)	13.1 ± 1.6(95)	16.3 ± 1.9(109)	16.8 ± 1.9(320)
Mold growth at home	19.0 ± 1.9(25)	16.0 ± 1.9(162)	14.6 ± 1.5(11)	12.7 ± 1.6(116)	17.6 ± 1.9(55)	16.6 ± 1.9(374)
Live in an urban area before 2 y age	21.9 ± 1.9(121)	18.2 ± 1.8(62)	13.2 ± 1.5(100)	12.8 ± 1.7(24)	17.3 ± 1.9(306)	15.4 ± 1.9(111)
Asthma-related symptoms						
Wheezing	40.5 ± 2.0(17)***	14.8 ± 1.7(170)	21.1 ± 1.7(6)**	12.5 ± 1.5(121)	24.8 ± 2.1(138)***	13.8 ± 1.7(291)
Doctors' diagnosed asthma	33.5 ± 2.2(17)***	15.1 ± 1.8(170)	20.4 ± 1.7(5)*	12.6 ± 1.6(122)	30.5 ± 2.0(57)***	15.2 ± 1.8(372)
Daytime breathlessness	21.6 ± 1.9(36)**	15.2 ± 1.8(151)	15.6 ± 1.7(29)**	12.1 ± 1.5(98)	20.5 ± 2.0(141)***	15.1 ± 1.9(388)
Nighttime breathlessness	20.0 ± 2.1(7)	16.1 ± 1.9(180)	14.0 ± 1.1(2)	12.8 ± 1.5(125)	20.8 ± 2.0(26)	16.4 ± 1.9(403)
Dry cough at night	21.3 ± 2.1(28)*	15.5 ± 1.8(159)	13.2 ± 1.5(18)	12.8 ± 1.6(109)	19.7 ± 2.1(98)**	15.9 ± 1.9(331)
Current rhinitis symptoms	22.8 ± 2.1(65)***	13.6 ± 1.6(122)	13.0 ± 1.7(43)	12.7 ± 1.5(84)	20.2 ± 2.1(192)***	14.3 ± 1.7(327)
Current eczema symptom	22.0 ± 2.1(6)	16.1 ± 1.9(181)	12.5 ± 1.3(4)	12.8 ± 1.6(123)	16.4 ± 2.0(31)	16.7 ± 1.9(398)
Pollen or pet allergy	29.5 ± 2.0(13)***	15.5 ± 1.8(176)	14.8 ± 1.4(5)	12.7 ± 1.6(122)	24.9 ± 2.1(46)***	15.9 ± 1.9(363)

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[a] Comparisons of FeNO values were made between 'Yes' and 'No' answers in each city in the randomly selected subjects as well as in the enriched subjects.

[b] Comparisons of FeNO values was made between the randomly selected subjects in Shanghai and Taiyuan.

Table 3 Univariate analyses of associations between selected risk factors and FeNO levels (GM ± GSD)(*n*), stratified by wheezing.

	Wheezing subjects (n = 138)[a]		Non-wheezing subjects†(n = 291)[a]	
	Yes	No	Yes	No
Center (shanghai = yes)	27.1 ± 2.2(100)*	19.7 ± 2.0(38)	14.8 ± 1.7(170)**	12.5 ± 1.5(121)
Boy	25.1 ± 2.2(84)	24.3 ± 2.1(54)	15.2 ± 1.6(146)*	12.6 ± 1.7(145)
Overweight and obesity	26.9 ± 1.7(25)	24.4 ± 2.2(113)	14.5 ± 1.6(39)	13.7 ± 1.7(252)
Parental asthma, allergy or eczema	31.5 ± 2.3(43) *	22.3 ± 2.0(95)	16.5 ± 1.9(35)*	13.5 ± 1.6(256)
Home ETS exposure	23.4 ± 2.1(102)	28.5 ± 2.3(31)	14.0 ± 1.7(214)	13.3 ± 1.6(65)
Doctors' diagnosed asthma	33.9 ± 2.0(45) **	21.3 ± 2.1(93)	20.7 ± 1.9(12)**	13.6 ± 1.6(279)
Daytime attacks of breathlessness	23.5 ± 2.1(93)	27.6 ± 2.2(45)	15.7 ± 1.7(48)	13.5 ± 1.7(243)
Nighttime breathlessness	22.7 ± 2.1(20)	25.2 ± 2.2(118)	15.4 ± 1.5(6)	13.8 ± 1.7(285)
Dry cough at night	22.8 ± 2.2(63)	26.6 ± 2.1(75)	15.1 ± 1.7(35)	13.6 ± 1.6(256)
Current rhinitis symptom	24.5 ± 2.2(103)	25.9 ± 2.1(35)	16.1 ± 1.9(89)***	12.9 ± 1.5(202)
Pollen or pet allergy	27.4 ± 2.2(31)	24.1 ± 2.1(107)	20.6 ± 1.7(15)**	13.5 ± 1.6(276)
Cat allergy	45.6 ± 2.0(11)*	23.5 ± 2.1(127)	19.4 ± 1.6(7)	13.7 ± 1.7(284)
Dog allergy	46.4 ± 2.0(10)*	23.6 ± 2.1(128)	19.7 ± 1.7(6)	13.7 ± 1.7(285)
Pollen allergy	25.6 ± 2.2(27)	24.6 ± 2.1(111)	21.6 ± 1.9(10)**	13.6 ± 1.6(281)

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[a] Comparisons of FeNO values were made between 'Yes' and 'No' answers in wheezing and non-wheezing subjects.

calculated, based on 95% CI of 10-log data. We excluded all children with doctors' diagnosed asthma, current wheeze, cumulative rhinitis, and cumulative eczema, using the same exclusion criteria as in the previous FeNO study from Beijing school children.[19] In the combined random sample (N = 165), the geometric mean (GM) value was 12.7 ppb (95% CI 5.5–29.1), with a GM of 14.3 ppb (95% CI of 5.9–34.7; N = 76) for boys and 13.2 ppb (95% CI 5.6–23.7; N = 89) for girls. In Shanghai, the GM was 13.2 ppb (95% CI 5.5–31.7; N = 65) and in Taiyuan 12.0 ppb (95% CI 5.7–25.2; N = 100). If making a less strict restriction in the random sample, only excluding subjects from the random sample with doctors' diagnosed asthma and current wheeze (N = 279), slightly wider confidence intervals were obtained (data not shown).

Discussion

In this study, the level of FeNO was significantly higher in Shanghai as compared to Taiyuan. Moreover male gender, parental asthma/allergy or eczema, doctors' diagnosed asthma, current respiratory symptoms (especially wheeze), current rhinitis and self-reported cat, dog and pollen allergy were significantly associated with increased FeNO values.

Epidemiological studies can be affected by selection bias and information bias. The participation rate in the initial questionnaire studies was high in both cities. Moreover the participation rate in the FeNO study was high. We enrolled a random sample of children from all classes, plus additional subjects with current wheeze not included in the random sample. This enabled us to get a higher power in the study on the investigation on the associations between respiratory symptoms and FeNO levels. In the random sample, the response rate was lower in Taiyuan as compared to Shanghai. This was mainly due to unexpected school activities in 2 schools in Taiyuan making it impossible for some students to participate because they were enrolled in other activities. It is less likely that this would cause any selection bias since the students that could not participate did not choose this by themselves, and participation or non-participation in unexpected activities was not related to respiratory health. Moreover, we had mostly the same significant associations when analyzing the random sample and the enriched total sample. Thus it is less likely that our results were influenced by selection bias. Information bias may be a problem in epidemiological studies, especially in studies relying on questionnaire data only. In this study, the children firstly answered the questionnaire and then participated in the clinical test, so questionnaire data could not be influenced by awareness of the FeNO values. Selection of a particular statistical model may also influence the results. However, we analyzed the data both by bivariate and multivariate analysis, and controlled for confounders by mutual adjustment in the multivariate analysis, and made the same type of analyses both in the random sample and the enriched total sample. The results from the different statistical analyses were mostly the same. Thus we do not believe that our conclusions were seriously influenced by selection bias, information bias, or use of a particular statistical model. However, the cross-sectional study design limits the possibility to draw conclusions about causal relationships.

Our measurements of air pollution outside the schools confirmed that the pollution levels were different between two cities, with higher levels of NO_2 in Shanghai and higher levels of SO_2 in Taiyuan. The main source of SO_2 is coal combustion, and the main source of NO_2 is traffic exhausts. The higher levels of FeNO in Shanghai as compared to Taiyuan may indicate that traffic exhaust is worse than coal combustion air pollution and this interpretation is in agreement with some other studies showing positive associations between traffic exhausts exposure and FeNO.[13,14] Since we only tested FeNO in this study, the conclusion that coal combustion air pollution is worse than traffic air

Table 4 Multiple linear regression analysis of FeNO levels (10 log-transformed) in the randomly selected group, total material, the wheezing and non-wheezing groups.[a]

	Randomly selected (n = 314)		Total material of wheezing and non-wheezing subjects (n = 429)		Wheezing subjects (n = 138)		Non-wheezing subjects (n = 291)	
	Antilog-β	P	Antilog-β	P	Antilog-β	P	Antilog-β	P
Center(Shanghai = 1,Taiyuan = 0)	1.21(1.08–1.35)	**0.001**	1.21(1.08–1.36)	**0.001**	1.34(1.00–1.80)	0.052	1.17(1.04–1.31)	**0.007**
Gender(boy = 1,girl = 0)	1.20(1.07–1.34)	**0.001**	1.14(1.02–1.28)	**0.021**	1.08(0.83–1.41)	0.575	1.19(1.06–1.33)	**0.002**
BMI	1.00(0.99–1.02)	0.686	1.01(0.99–1.02)	0.496	1.01(0.97–1.05)	0.761	1.00(0.99–1.02)	0.568
Parental asthma, allergy or eczema	1.12(0.94–1.32)	0.198	1.17(1.00–1.36)	**0.044**	1.25(0.94–1.67)	0.118	1.04(0.87–1.24)	0.643
Doctors' diagnosed asthma	1.36(1.07–1.73)	**0.013**	1.42(1.18–1.71)	**<0.001**	1.49(1.11–2.00)	**0.009**	1.35(1.01–1.79)	**0.039**
Wheezing or whistling in the past 12 months	1.82(1.42–2.33)	**0.000**	1.46(1.25–1.69)	**<0.000**	–	–	–	–
Daytime attacks of breathlessness	1.10(0.95–1.28)	0.194	1.03(0.89–1.18)	0.717	–	–	–	–
Dry cough at night	0.98(0.83–1.15)	0.790	0.90(0.78–1.05)	0.175	–	–	–	–
Current rhinitis symptoms	1.15(1.01–1.30)	**0.029**	1.11(0.98–1.26)	0.108	0.87(1.65–1.16)	0.331	1.20(1.06–1.35)	**0.004**
Pet or pollen allergy	1.32(1.03–1.68)	**0.027**	1.07(0.88–1.29)	0.515	0.93(0.67–1.28)	0.652	1.32(1.02–1.70)	**0.036**

[a] Factors significantly associated with FeNO levels in the univariate analysis were included in the multivariate linear regression model. For wheezing (n = 138) and non-wheezing subjects (n = 291), wheezing, daytime attacks of breathlessness and dry cough at night in the past 12 months were not added in the model. $P < 0.05$ values marked in bold.

pollution may not be true for other types of respiratory effects. Moreover, the difference in FeNO levels could be due to different prevalence of respiratory disease or allergies. We did not have information on allergy to house dust mites or moulds. House dust mite allergy is a major allergy in China.[24] It could be expected that the indoor levels of house dust mite allergens and moulds were higher in Shanghai than in Taiyuan since the climate in Shanghai is more humid. Even if we cannot be sure which factors that caused the observed difference in FeNO, our findings supported the conclusion that the higher level of asthma in the more developed part of China is real,[3] and not due to differences in diagnostic criteria.

The normal FeNO value (12.7ppb, 95% CI 5.9—34.7ppb) in healthy children in our study (age 12—14 y) was well in agreement with the previous study from school children in Beijing, using the same definition of healthy children.[19] They found a GM value of 11.22 ppb in healthy children aged 11—14 y (95% CI 4.17—30.20). The GM value in healthy children in our study did not differ between Shanghai and Taiyuan, but was higher in boys as compared to girls. This gender difference was in agreement with previous data.[4,25] In the ATS clinical practice guideline for interpretation of FeNO, cut points rather than normal values are preferred. They suggest cut points at 25 ppb (low FeNO) and 50 ppb (high Fe NO) for adults, and 20 ppb and 35 ppb as cut points for children. The values between are classified as intermediate FeNO levels. At low FeNO, eosinophilic airway inflammation is unlikely, and at high FeNO level, eosinophilia is likely. Intermediate FeNO values should be interpreted with caution and with reference to the clinical context.[25] We hereby presented data using both sets of cut point (Table 6), since the cut point of 25 ppb for elevated FeNO is well established, but because our participants were children, we still based our conclusions on the cut points for children. If we analyzed the random samples only and used the cut points suggested for children, high FeNO (>35 ppb) was 5 times more common in Shanghai as compared to Taiyuan (11.3% vs.2.4%). Moreover, an intermediate or higher FeNO (>=20ppm) was 2 times more common in Shanghai as compared to Taiyuan (32.3% vs. 14.2%). This indicated that eosinophilic airway inflammation was more common in Shanghai, and also suggested that the differences in asthma and asthmatic symptoms between the two cities observed in questionnaire studies were not likely due to different diagnostic criteria for asthma.[18,21]

The prevalence of doctors' diagnosed asthma in the random sample was relatively low especially in Taiyuan. In the total material, 57 reported doctors' diagnosed asthma. The low number could be partly due to the exclusion of children with current asthma medication mainly in Shanghai. Among the children with doctors' diagnosed asthma, all without asthma medication, high FeNO values (>35ppb) indicating eosinophilic inflammation were found in 44% of the children. Low FeNO values (<20ppb) indicating other causes of asthma were found in 32%. This suggested that eosinophilic inflammation is common in children with asthma diagnosis but no current asthma medication in China, but other causes of the asthma may be equally common. Currently, the clinical standard of asthma diagnosis in mainland of China is mainly based on asthma related symptoms or combined with BHR test or lung

Table 5 Associations between FeNO levels and asthmatic or allergic diseases and symptoms, analysed by multiple logistic regression (OR,95%CI).[a]

	Model I (n = 314)	Model II (n = 429)	Model III (n = 291)	Model IV (n = 237)	Model V (n = 383)
Doctors' diagnosed asthma	1.45 (1.22–1.73)***	1.32 (1.19–1.47)***	1.33 (1.05–1.67)*	1.27 (1.04–1.55)*	1.28 (1.13–1.45)***
Wheeze	1.71 (1.40–2.10)***	1.35 (1.23–1.47)***	–	1.65 (1.37–2.00)***	1.37 (1.24–1.51)***
Daytime breathlessness	1.25 (1.11–1.41)***	1.17 (1.08–1.26)***	1.18 (1.02–1.36)*	1.19 (1.04–1.37)*	1.20 (1.10–1.30)***
Dry cough at night	1.15 (1.02–1.31)*	1.08 (0.99–1.17)	1.11 (0.95–1.31)	1.09 (0.92–1.31)	1.12 (1.02–1.22)*
Current rhinitis symptom	1.25 (1.12–1.39)***	1.18 (1.09–1.27)***	1.22 (1.08–1.38)**	–	1.16 (1.07–1.26)***
Pollen or pet allergy	1.27 (1.07–1.52)**	1.17 (1.16–1.30)**	1.32 (1.07–1.64)*	1.16 (0.92–1.46)	–

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. ORs were calculated per one unit of FeNO on the 10 log-transformed scale.
Model I: includes only randomly selected subjects (n = 314).
Model II: includes all subjects (wheezing/non-wheezing enriched sample) (n = 429).
Model III: includes only non-wheezing subjects (n = 291).
Model IV: includes only subjects without current rhinitis (n = 237). Model V: includes only subjects without pollen or pet allergy (n = 383).
[a] Each model was controlled for center, gender, BMI and parental asthma, allergy or eczema.

function test.[26] Evaluation of airway inflammation level is very rare in asthma diagnosis in China.

In our study we added subjects with current wheeze, and the power to study the association between wheeze and FeNO was good. Wheeze is one of the most common respiratory symptoms used to monitor asthma in epidemiological studies. I has been questioned if the concept of wheeze is understood well in all parts of the world, e.g. in Asian countries. However we found that wheeze was strongly associated with FeNO, and one third (33.8%) of all subjects reporting current wheeze had a high FeNO, indicating eosinophilia. However, 41.9% of the wheezing subjects had a low FeNO, indicating that almost half of the children had wheeze that was caused by other factors than eosinophilic airway inflammation. Current wheeze was associated with some personal factors (male gender, BMI, and parental asthma/allergy or eczema) as well as dampness and moulds in the current dwelling. In contrast FeNO was related to male gender and parental asthma/allergy or eczema, only. This may indicate that wheeze associated with BMI or indoor dampness and moulds in the dwelling is caused by other mechanisms than eosinophilic inflammation in Chinese school children.

Other asthmatic symptoms such as daytime attacks of breathlessness and dry cough at night were associated with FeNO in the initial analysis in the random sample, but if restricting the analysis to non-wheezing subjects, the associations were no longer significant. This suggests that wheeze is more closely related to FeNO than the other asthmatic symptoms investigated in this study.

Current rhinitis was very common and associated with higher FeNO values. The associations remained significant in the multivariate analysis even after excluding either wheezing subjects, or those reporting pollen or furry pet allergy. This is in agreement with some previous studies showing that allergic rhinitis is associated with elevated levels of FeNO.[8] We did not have data on bronchial hyperresponsiveness (BHR) in this study and could not study to what extent BHR influenced the FeNO levels. In a clinical study in Chinese patients, 76% of non-asthmatic allergic rhinitis patients had high BHR.[27] The association between allergic rhinitis and FeNO or BHR is most likely due to

Table 6 Proportions of subjects (%, (n)) in the random sample with low, intermediate and high FeNO, by two different cut points.[a]

FeNO	Total (n = 313)	Taiyuan (n = 127)	Shanghai (n = 186)
Cut points: 20–35 ppb (for children)			
<20ppb (Low value)	75.1 (235)	85.8 (109)	67.7 (126)
20 ≤ FeNO ≤35 ppb (Intermediate)	17.3 (54)	11.8 (15)	21.0 (39)
>35 (High value)	7.7 (24)	2.4 (3)	11.3 (21)
Chi-x^2 test	Chi x^2 test $P < 0.001$		
Cut points: 25–50 ppb (for adults)			
<25ppb (Low value)	83.7 (262)	92.9 (118)	77.4 (144)
25 ≤ FeNO≤50 ppb (intermediate)	11.8 (37)	6.3 (8)	15.6 (29)
>50 ppb (High value)	4.5 (14)	0.8 (1)	7.0 (13)
Chi-x^2 test	Chi x^2 test $P < 0.001$		

[a] One smoker (from Shanghai) was excluded from the analysis.

a general airway inflammation affecting both the upper and lower airway ("The united airway concept"). The association between self-reported pollen or pet allergy and elevated FeNO in our study is in agreement with other studies diagnosing IgE sensitization by allergy testing.[4,25] However, one limitation of our study is that we did not have information on allergy to house dust mites or cockroach, the most common IgE mediated allergies in China.[28] This means that some subjects among those without any pollen or pet allergy could have allergy and allergic rhinitis from other indoor allergens.

In conclusion, male gender, current wheeze, doctors' diagnosed asthma, parental asthma/allergy, current rhinitis, pollen allergy and furry pet allergy were associated with increased FeNO. The lower level of FeNO in Taiyuan is in agreement with previous questionnaire studies showing lower prevalence of asthma and allergy in this city as compared to Shanghai. It suggested that when taking FeNO measurement as a screening tool for asthmatic children, other diseases, e.g. allergic rhinitis, need to be considered. China has the largest population in the world, but we found few publications on FeNO in children from a community population.[12,19,28]

This is the first study from mainland of China investigating FeNO levels in relation to asthma, rhinitis, dermatitis and early childhood factors and home environmental exposures in cities with different pollution levels.

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Conflict of interest statement

The authors have no conflicting financial interests.

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