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Reference values for exhaled nitric oxide in healthy children aged 6–18 years in China: a cross-sectional, multicenter clinical study

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Abstract

Background The reference values of eNO have certain differences among people of different countries and races. We aimed to obtain the reference value of eNO in healthy children and adolescents (6–18 years old) in China and to explore the associations between the reference values with ages, gender, heights, BMI, and regions.

Methods We measured $FeNO_{50}$ levels in 5949 healthy Chinese children and adolescents, $FeNO_{200}$ and CaNO levels in 658 participants from 16 provinces of 7 administrative areas in China aged 6–18. All persons were studied after obtaining informed consent from children and their parents.

Results The mean $FeNO_{50}$ of 5949 Chinese children and adolescents aged 6–18 years was 14.1 ppb, with a 95% confidence interval of 1-38.1 ppb. The mean $FeNO_{200}$ of 658 persons was 6.9 ppb with a 95% upper confidence interval of 15.0 ppb, and the mean CaNO was 3.0 ppb with a 95% upper confidence interval of 11.2 ppb. In the 6–11 age group, age and height were correlated with the logarithm of $FeNO_{50}$ ($P<0.001$, $P<0.05$). There was no significant correlation between the logarithm of $FeNO_{200}$ and gender, age, height and BMI (all $P>0.05$). The logarithm of CaNO was correlated with gender ($P<0.05$). In the 12–18 age group, gender, height, and region were correlated with the logarithm of $FeNO_{50}$ (all $P<0.001$). There was only a weak correlation between the logarithm of $FeNO_{200}$ and height ($P<0.001$). The logarithm of CaNO was negatively correlated with age ($P<0.05$).

Conclusions Higher $FeNO_{50}$, $FeNO_{200}$ and CaNO values were found in healthy children and adolescents in China compared with foreign reports, and is affected by age, height, gender, and region. This study provides useful references for clinical application of eNO in children, especially Asian children.

Keywords $FeNO_{50}$, $FeNO_{200}$, CaNO, Reference values, Children and adolescents

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Background

Nitric oxide (NO), a gas signaling molecule of endogenous origin, is synthesized within the airway epithelial cells and its production is enhanced during eosinophilic inflammatory conditions. Fractional Exhaled Nitric Oxide (FeNO) has been acknowledged as a biomarker for type 2 inflammation in the airway, which plays a crucial role in bronchial hyper-responsiveness, mucus secretion, goblet cell hyperplasia, T-helper type 2 polarization, as well as allergic and eosinophilic inflammation [1]. In 2005, the American Thoracic Society (ATS) and the European Respiratory Society (ERS) jointly published the technical guidelines for the quantification of exhaled NO [2]. In 2011, the ATS endorsed the use of FeNO as a diagnostic tool for eosinophilic airway inflammation and as a reference for the administration of inhaled corticosteroids [3]. Similarly, the Global Initiative for Asthma (GINA) guidelines, the National Institute for Health and Care Excellence (NICE) guidelines, and other national asthma guidelines also advocate for the utilization of FeNO in the diagnosis and assessment of asthma in both adults and children [4–7].

FeNO serves as an indicator of eosinophilic airway inflammation, with its concentration varying across different segments of the airway and detectable at various flow rates, thereby providing insights into the inflammation present in the large airway, small airway, and upper airway [8]. The 2011 AST guidelines have suggested the use of $FeNO_{50}$ as a reference value for assessing NO levels in children. However, it is important to note that $FeNO_{50}$, which measures NO levels at a low flow rate of 50 ml/s, only reflects the NO levels in the large airways. Recent clinical studies have shown that patients with asthma often experience inflammation in the small airways. As a result, current guidelines recommend the use of $FeNO_{200}$ and CaNO as markers for small airway inflammation [8, 9]. However, there is still a lack of established reference values for these small airway inflammation indicators ($FeNO_{200}$, CaNO).

The determination of reference values for exhaled NO (eNO) and the identification of influencing factors are crucial in the assessment of asthma. Despite the recommendation of ATS standards for the reference value of $FeNO_{50}$, variations in the reference value of eNO persist among individuals from different countries and races. Notably, studies conducted in North America and Europe have provided insights into the normal values of $FeNO_{200}$ and CaNO as markers of small airway inflammation, yet there is a lack of description regarding these values in Asian children.

Hence, the Shanghai Children's Medical Center assumed the leading role in a collaborative effort involving 19 branch centers affiliated with the Respiratory department of children's hospitals or the Pediatric department of general hospitals across 16 provinces in China. This multi-center study, conducted from May 2018 to May 2021, aimed to establish the baseline levels of eNO in healthy children aged 6–18 years. The ultimate objective was to ascertain the normal range of eNO in Chinese children, thereby furnishing a valuable reference for the clinical diagnosis and utilization of eNO in pediatric healthcare settings in China and potentially throughout Asia.

Materials and methods

Study participants and design

This is a cross-sectional study of children and adolescents aged 6–18 in China from May 2018 to May 2021. Informed consent was obtained from the parents or legal guardians of the children. The inclusion criteria were as follows: ① The ethnicity is Han, and the age range is 6 to 18 years old; ② Born in China, both parents are Chinese Han nationality; ③ No history of respiratory tract infections within 4 weeks before; ④ No history of recurrent respiratory tract infections; ⑤ No allergic diseases such as asthma and rhinitis; ⑥ No family history of asthma; ⑦ No neurological disorders; ⑧ Normal intellectual development; ⑨ No smoking in the family environment; ⑩ No congenital lung and heart disease. A total of 8000 children were enrolled in the study. This study was approved by the Ethics Committee of Shanghai Children's Medical Center (Ethics No: SCMCIRB-K2017007), and each subcenter follows the master research unit ethics. Clinical Trial: ChiCTR1800019029 (Registration date was October 22, 2018).

Here, 20 centers from 16 provinces, seven areas in China (North, East, Northeast, Middle, South, Southwest, and Northwest), participated in this study. Of the 400 healthy children were recruited from each center and divided into two age groups: group I (age: 6–11 years old) had 200 cases, with 100 males and 100 females; Group II (age: 12–18 years old) had 200 cases, with 100 males and 100 females. Each center recruited healthy volunteers through local primary and secondary schools. After obtaining the informed consent of children and parents, the study was conducted.

Procedures

Physical growth index measurement

All of the subjects' heights and weights were recorded by fixed staff. The participants removed their shoes and wore shirts or light sweaters. Their weight was determined by scale that was accurate to 0.1 kg and their height by a vertical altimeter that was accurate to 0.1 cm. The children's birth dates and genders were noted simultaneously.

Determination of exhaled NO

All enrolled children were screened for healthy children by spirometry and then tested for exhaled NO. $FeNO_{50}$ was measured in children from seven areas of China, $FeNO_{200}$ and CaNO were measured in children from East China. The eNO was measured by the Nacoulomb breath analyzer. The subjects were tested for eNO according to the 2005 ATS/ERS and the 2017 ERS guidelines [1, 8]. Eating, strenuous exercise, or pulmonary function tests were banned 1 h before the test. Before the test, the operator will explain the test method and precautions to the subject, consult and fill in the subject's information (including age, sex, height and weight).

The test was performed with the subject in a seated position, holding the inspiratory filter, exhaling the remaining air, then covering the mouth with the filter, inhaling first and then exhaling at a flow rate of 50 ml/s and 200 ml/s, respectively, as required by the test. The values of $FeNO_{50}$ and $FeNO_{200}$ were recorded in ppb. Two tests were attempted for each subject at a single flow rate with an error of less than 10%, and unsuccessful attempts were recorded as test failures. CaNO values were calculated from $FeNO_{50}$ and $FeNO_{200}$.

According to the 2017 ERS guideline, the calculation of CaNO requires exhalation detection at 3 flow rates. However, this method is operationally difficult in younger children. In 2014, Peter J Barnes suggested that a low flow rate (50 ml/s) and a high flow rate (200 ml/s) could be used to differentiate NO in the large and small airways [9]. Therefore, the method of CaNO calculation was simplified using a two-compartment linear model in this study. To ensure the accuracy of the instruments, two standard gas mixtures with NO concentrations of 60 ppb and 250 ppb were used to test and calibrate the instruments before the start of the project in each subcenter.

Statistical analysis

The Kolmogorov-Smirnov method was used to test the normality of $FeNO_{50}$, $FeNO_{200}$ and CaNO. Data that failed the normality test were logarithmically transformed into data with a normal distribution. $FeNO_{50}$, $FeNO_{200}$ and CaNO were skewed distributions, so the normal reference values were calculated using the 95% upper limit value in the logarithmic state, and the actual values were calculated after taking the logarithm, corresponding to the 95% upper limit value, which is the upper limit of the normal values of the measured values.

Multiple stepwise regression and multiple linear regression analysis were performed on factors that may affect $FeNO_{50}$, $FeNO_{200}$ and CaNO: age, sex, height, Body-Mass-Index (BMI), and region (for $FeNO_{50}$ only) as independent variables. VIF was used to test interactions between variables. Weight was not counted in the analysis because it was covariate with BMI. For gender comparisons between males and females, t-tests were used for comparisons between groups conforming to normally distributed data, and nonparametric tests were used for comparisons between groups with nonnormally distributed data. All statistical analyses were performed using SPSS 22.0(IBM, Armonk, NY, USA). $P<0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 8000 children were completing the enrollment questionnaire. The reasons for exclusion were (1) incomplete questionnaire ($n=231$), (2) failure to fall within the predetermined age range ($n=13$), (3) having a history of eating within 1 h before measurement ($n=344$), (4) having strenuous exercise within 1 h before measurement ($n=208$), (5) inability to complete the $FeNO_{50}$ measurement ($n=467$) and spirometry($n=582$), (6) abnormal pulmonary ventilation function ($n=206$). Finally, a total of 5949 healthy children were included in the statistical analysis (Fig. 1). Subject characteristics, including the total study sample with eNO measurements, are presented in Table 1. $FeNO_{50}$ measurements were performed in 5949 children. Of the 3534 children (1881 males and 1653 females) aged 6–11 years, and 2415 children (1220 males and 1195 females) aged 12–18 years. A total of 800 children from two centers in East China participated in $FeNO_{200}$/CaNO determination, and only 658 cases (359 males and 299 females) completed the determination of $FeNO_{200}$/CaNO. 345 children (199 males and 146 females) aged 6–11 years, and 313 children (160 males and 153 females) aged 12–18 years.

Normal ranges of $FeNO_{50}$, $FeNO_{200}$ and CaNO in healthy children

In this study, the geometric mean of $FeNO_{50}$ in 5949 children aged 6–18 years was 14.1ppb (P_{25}-P_{75}:13.9-14.3ppb), and the 95% upper limit was 38.1ppb. The 95% upper limit of $FeNO_{50}$ in children aged 6–11 years was 38.1ppb, and the geometric mean was 13.1ppb (P_{25}-P_{75}:12.9-13.4ppb), which respectively were 38.2ppb and 15.9ppb (P_{25}-P_{75}:13.4-13.9ppb) in children aged 12–18 years (Fig. 2).

The range of $FeNO_{200}$ in 658 children aged 6–18 years was 1-31ppb, with the geometric mean being 6.9ppb (P_{25}-P_{75}:6.9-7.2ppb) and the 95% upper limit being 15.0ppb. The 95% upper limit of $FeNO_{200}$ in children aged 6–11 years was 16.0ppb, and the geometric mean was 6.6ppb (P_{25}-P_{75}:6.3-7.0ppb), which respectively were 15.0ppb and 7.3ppb (P_{25}-P_{75}:6.3-7.0ppb) in children aged 12–18 years (Fig. 3).

The range of CaNO in 658 children aged 6–18 years was 0.5-21.1ppb, with the geometric mean being 3.0ppb (P_{25}-P_{75}:2.9-3.2ppb) and the 95% upper limit was

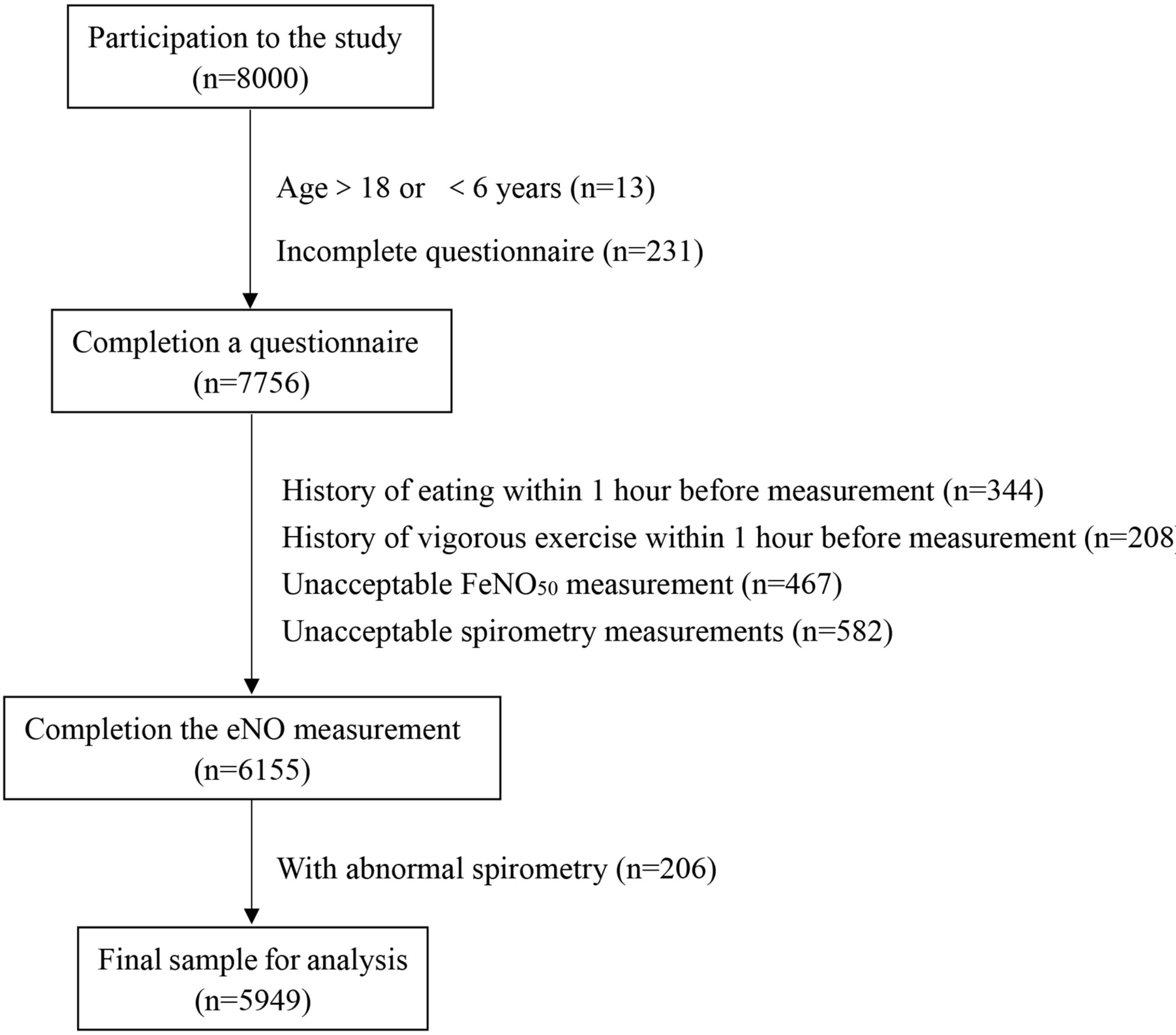

Fig. 1 Schematic presentation of the recruitment of healthy children

Table 1 The demographic data of the participants

	$FeNO_{50}$			$FeNO_{200}$/CaNO		
	All (*N* = 5949)	**6–11 years (*N* = 3534)**	**12–18 years (*N* = 2415)**	**All (*N* = 658)**	**6–11 years (*N* = 345)**	**12–18 years (*N* = 313)**
Age, years, M (P_{25}, P_{75})	10.0 (8.0, 13.0)	9.0 (7.0, 10.0)	14.0 (13.0, 16.0)	11.0 (9.0, 15.0)	9.0 (8.0, 10.0)	15.0 (13.0, 17.0)
Sex						
Male	3101 (52.1%)	1881 (53.2%)	1220 (50.5%)	359 (54.6%)	199 (57.7%)	160 (51.1%)
Female	2848 (47.9%)	1653 (46.8%)	1195 (49.5%)	299 (45.4%)	146 (42.3%)	153 (48.9%)
Height, cm, M (P_{25}, P_{75})	147.0 (133.0, 161.0)	135.0 (128.0, 144.0)	163.0 (157.0, 170.0)	150.5 (137.0, 165.0)	137.0 (131.0, 144.0)	165.0 (159.0, 172.0)
Weight, kg, M (P_{25}, P_{75})	39.9 (28.0, 53.0)	30.0 (25.0, 38.0)	54.0 (47.0, 62.8)	45.0 (32.0, 58.0)	33.0 (27.0, 41.0)	58.0 (50.0, 67.5)
BMI, kg/m^2, M (P_{25}, P_{75})	18.0 (15.6, 20.8)	16.3 (14.9, 18.9)	20.0 (18.1, 22.6)	19.2 (16.6, 22.0)	17.4 (15.5, 20.3)	20.7 (18.7, 24.0)

11.2ppb. The 95% upper limit of CaNO in children aged 6–11 years was 12.4ppb, and the geometric mean was 3.6ppb (P_{25}-P_{75}:3.3-4.0ppb), which respectively were 8.8ppb and 2.8ppb (P_{25}-P_{75}:2.6-3.1ppb) in children aged 12–18 years (Fig. 4).

Factors affecting $FeNO_{50}$ values

Only taking into account independent variables, the logarithm of $FeNO_{50}$ was significantly correlated with age, height, and BMI in children aged 6 to 18 (Figs. 5A and 6A, all $P<0.001$). $FeNO_{50}$ in males was significantly

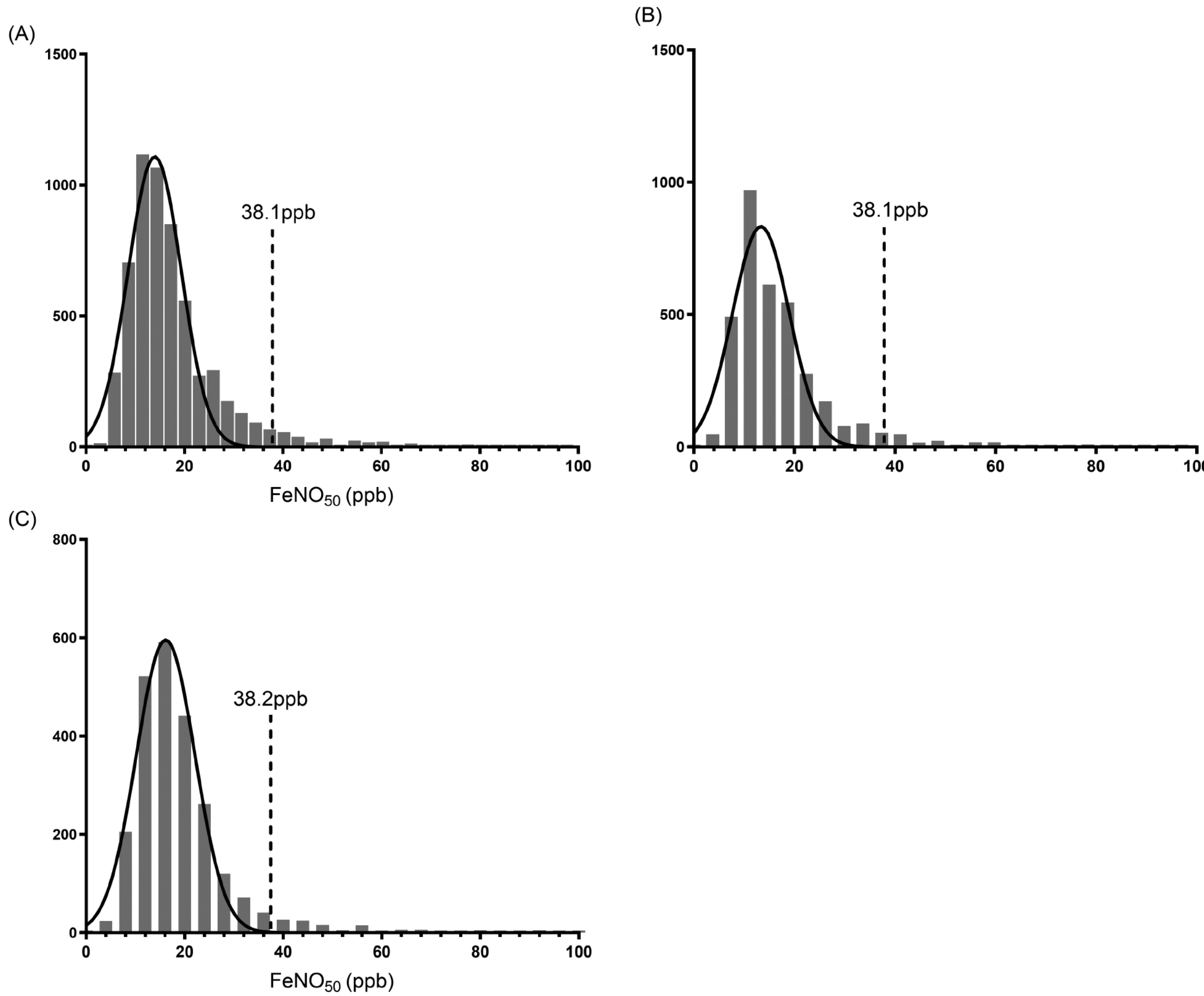

Fig. 2 Distribution of $FeNO_{50}$. **(A)** $FeNO_{50}$ in children aged 6–18 years; **(B)** $FeNO_{50}$ in children aged 6–11 years; **(C)** $FeNO_{50}$ in children aged 12–18 years

higher than that in females, and there were significant differences among different regions (Fig. 7A, $P<0.05$). According to the results of multivariate regression analysis, the logarithm of $FeNO_{50}$ has a significant relationship with height, region, and gender (all $P<0.05$), but not to age and BMI in children aged 6–18 years (all $P>0.05$).

For children aged 6–11 years, the logarithm of $FeNO_{50}$ was significantly correlated with age, height and BMI when only independent factors were considered. There was no significant difference in $FeNO_{50}$ values between males and females, but there were significant differences in different regions (Fig. 7B, $P<0.05$). The results of multivariate regression analysis showed that age and height were significantly correlated with the logarithm of $FeNO_{50}$, while gender, BMI and regions were not (Table 2).

For children and adolescents aged 12–18 years old, the logarithm of $FeNO_{50}$ was significantly correlated with height, but not with age or BMI when only independent factors were considered. The $FeNO_{50}$ value of males was significantly higher than that of females, and there were significant differences in different regions (Fig. 7C, $P<0.05$). The results of multivariate regression analysis showed that the logarithm of $FeNO_{50}$ was significantly correlated with gender, height and region, but not with age and BMI (Table 3).

Factors affecting $FeNO_{200}$ values

Only taking into account independent variables, the logarithm of $FeNO_{200}$ was significantly correlated with height (Fig. 6B, $P<0.05$), but not with age and BMI (Fig. 5B). $FeNO_{200}$ in males was significantly higher than that in females ($P<0.05$). Multivariate regression analysis showed that the logarithm of $FeNO_{200}$ was correlated with height and BMI, but not with gender and age.

For children aged 6–11 years, there was no significant correlation between the logarithm of $FeNO_{200}$ and age, height and BMI when independent factors

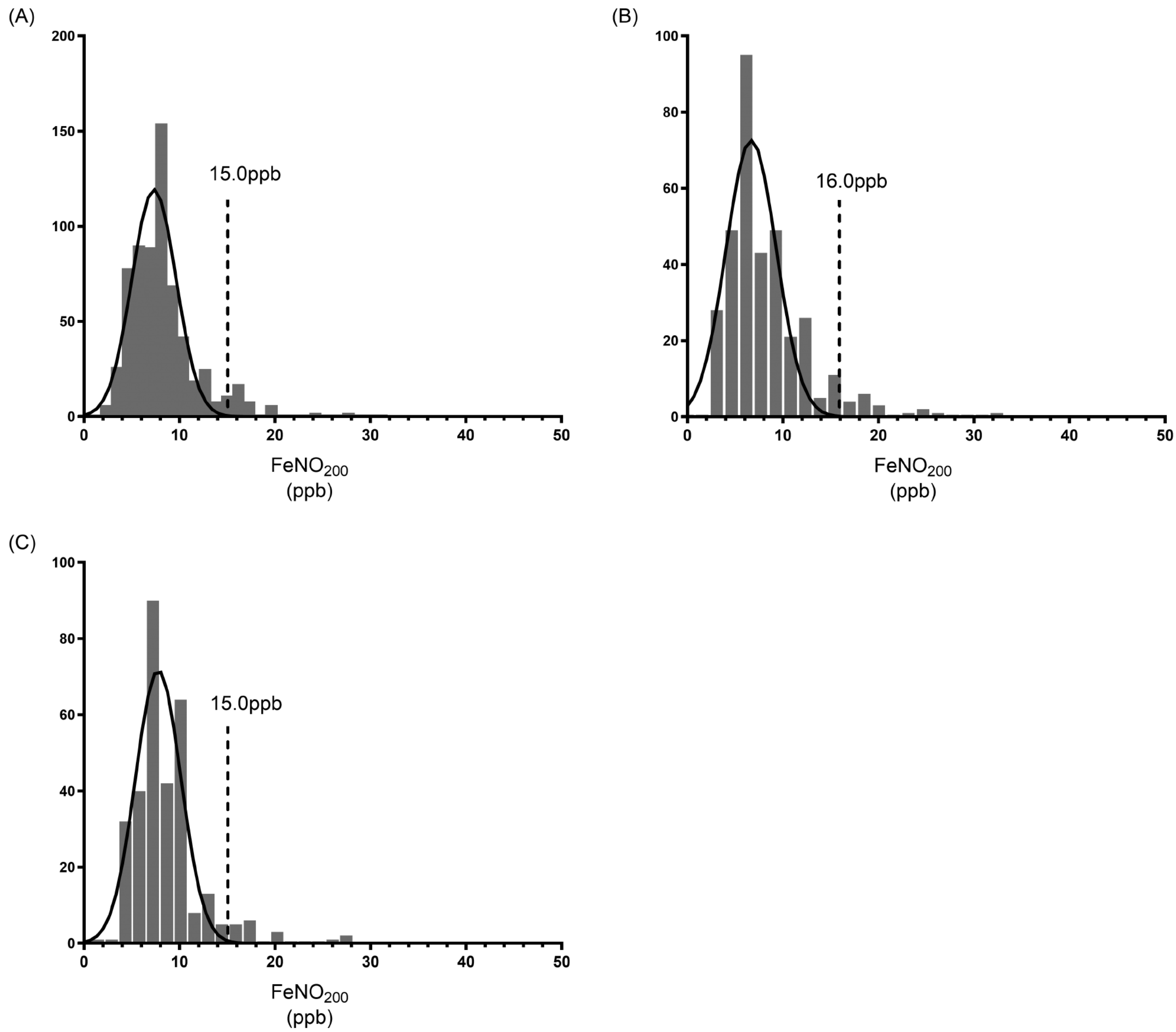

Fig. 3 Distribution of $FeNO_{200}$. **(A)** $FeNO_{200}$ in children aged 6–18 years; **(B)** $FeNO_{200}$ in children aged 6–11 years; **(C)** $FeNO_{200}$ in children aged 12–18 years

were considered. There was no significant difference in $FeNO_{200}$ values between males and females (P>0.05). The results of multivariate regression analysis showed that gender, age, height and BMI were not significantly correlated with the logarithm of $FeNO_{200}$ (Table 2).

For children and adolescents aged 12–18 years, the logarithm of $FeNO_{200}$ was significantly correlated with height, but not with age and BMI when only independent factors were considered. The $FeNO_{200}$ value of males was significantly higher than that of females. Multivariate regression analysis showed that for the logarithm of $FeNO_{200}$ was significantly correlated with height, but not with age, gender, and BMI (Table 3).

Factors affecting CaNO values

For children aged 6–18 years, the logarithm of CaNO was negatively correlated with age and height when only independent factors were considered (Figs. 5C and 6C, all $P<0.05$) and had no correlation with BMI (P>0.05). There was no significant difference between males and females in the values of CaNO. Multivariate regression analysis showed that the logarithm of CaNO was negatively correlated with height, but not with gender, age and BMI.

For children aged 6–11 years, there was no significant correlation between the logarithm of CaNO and age, height and BMI when independent factors were considered. There was no significant difference in CaNO values between males and females (P>0.05). Multivariate regression analysis showed that the logarithm of CaNO was related to gender, but not age, height or BMI (Table 2).

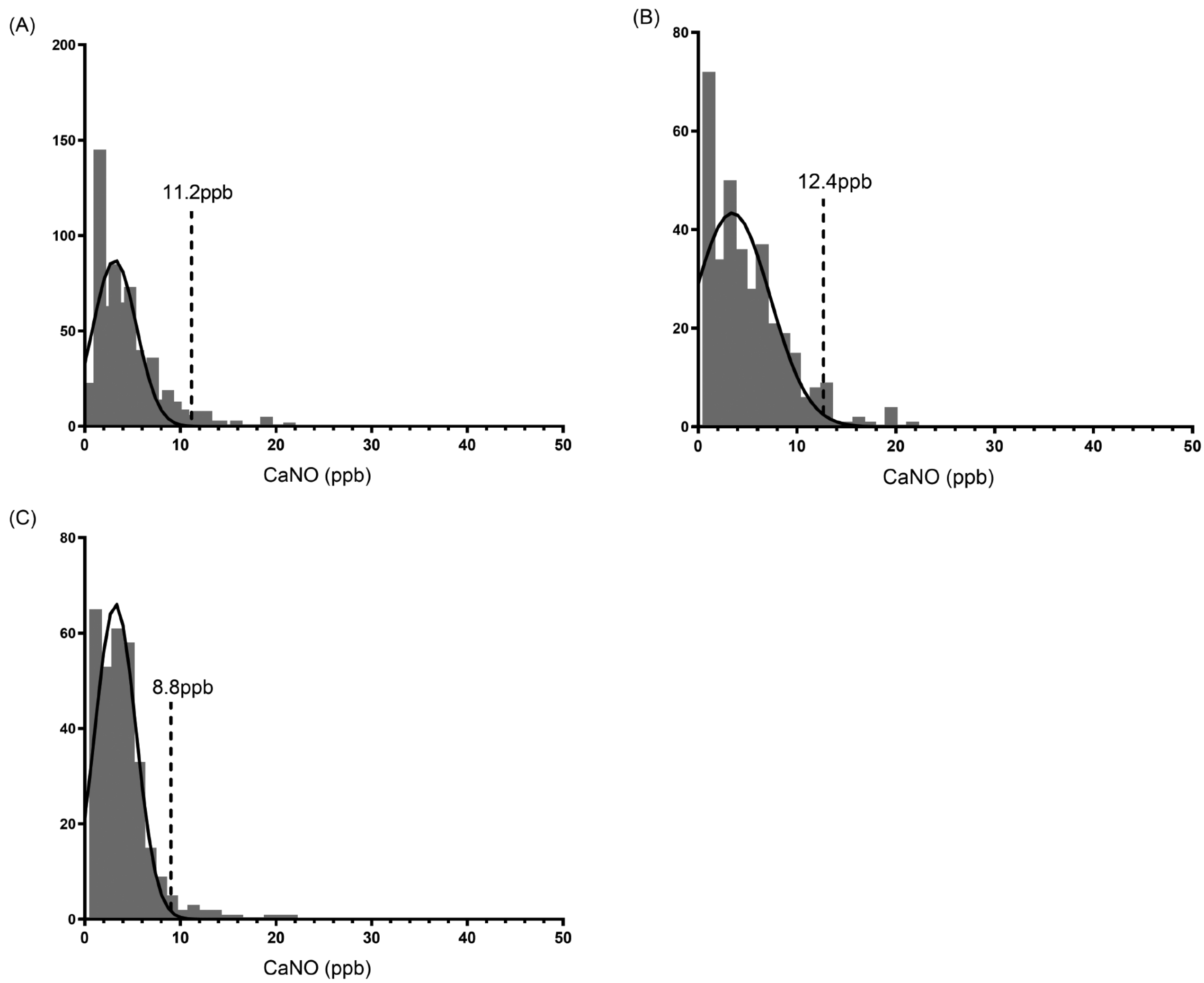

Fig. 4 Distribution of CaNO. **(A)** CaNO in children aged 6–18 years; **(B)** CaNO in children aged 6–11 years; **(C)** CaNO in children aged 12–18 years

For children and adolescents aged 12–18 years, the logarithm of CaNO was negatively correlated with age, but not with height and BMI when only independent factors were considered. There was no difference in CaNO values between males and females (P>0.05). Multivariate regression analysis showed that the logarithm of CaNO was negatively correlated with age, but not with height, gender, and BMI (Table 3).

Discussion

In assessing airway inflammation, diagnosing asthma, assessing asthma control, directing treatment, and estimating the likelihood of recurrence, the detection of eNO is crucial according to the ERS [8]. The FeNO combined with GINA guideline group helps to reduce the daily dose of ICS and treatment costs [10]. However, given the differences in race, location, region and environment, the reference value of eNO varies. Current international guidelines are based predominantly on data collected from white populations. If the interpretation is based on unsuitable reference values, it will cause incorrect results and mislead clinicians. Therefore, it is crucial to clarify the normal reference values held by Chinese children.

To our knowledge, this study is the largest sample research to explore the normal reference values of eNO in healthy children until now. The results showed that the geometric mean of $FeNO_{50}$ aged 6–18 years was 14.1 ppb with an upper 95% confidence interval of 38.1 ppb, which is similar to the previous study for 9–22 years people in China [11], and to those in the United States and Canada, but higher than those in Europe (Italy, Finland, Spain, Slovakia, and France) (e-Table 1) [12–21]. According to the study, Asian and African populations have higher $FeNO_{50}$ values than Whites. This.

may be the reason for this phenomenon because the United States and Canada are large immigrant countries with a larger population of other races besides Whites. The geometric mean of $FeNO_{200}$ in 658 children was 6.9

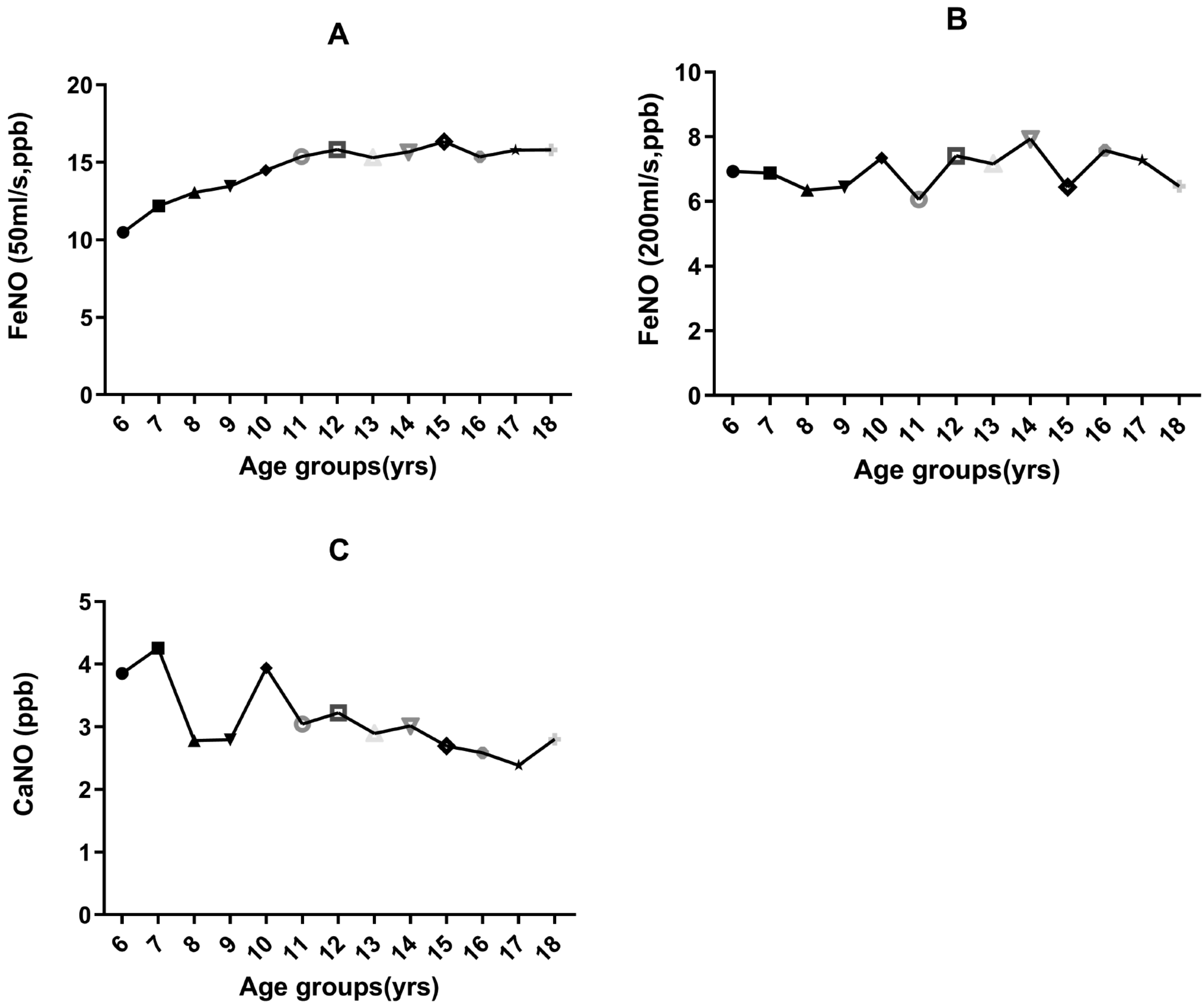

Fig. 5 $FeNO_{50}$, $FeNO_{200}$ and CaNO values in children of all ages

ppb, with an upper 95% limit of 15.0 ppb and the mean of CaNO was 3.0 ppb, with an upper 95% limit of 11.2 ppb, which are higher than the previous studies from the UK, Finland, USA, and Spain (e-Table 2) [22–27]. The reasons may be the same as $FeNO_{50}$, $FeNO_{200}$ and CaNO are higher in populations of Asian origin than in European populations, but also need a large sample clinical study to verify.

Age and height are known to be associated independently with FeNO values [28, 29]. In this study, we found that $FeNO_{50}$ was positively correlated with age and height in children aged 6–11 years, $FeNO_{50}$ and $FeNO_{200}$ were positively correlated with height in children aged 12–18, VIF for age and height were below 3, threshold for collinearity diagnostics. This showed that there is no multicollinearity effect between age and height. Those findings are consistent with the previous studies. The age-dependent eNO may be due to two reasons. One is that the airway surface area increases with age, increasing NO diffusion in the lungs [30]. Second, repeated immune stimulation during growth increased the formation of inducible NO synthase [31]. In children aged 12–18, CaNO was inversely associated with age, similar to a previous study, which found that CaNO decreased with age in people under 20 years old. The reason for this correlation needs further investigation.

Gender, as one of the important influencing factors of eNO, our study found that $FeNO_{50}$ and $FeNO_{200}$ in males were significantly higher than those in females in children aged 12–18, and CaNO was significantly higher in males than females for children aged 6–11. The findings are consistent with some clinical studies [32, 33]. The difference in airway surface area and diameter between males and females may be the cause of the gender disparity. The small airway space in females leads to different NO diffusion, thus the eNO values of female children are

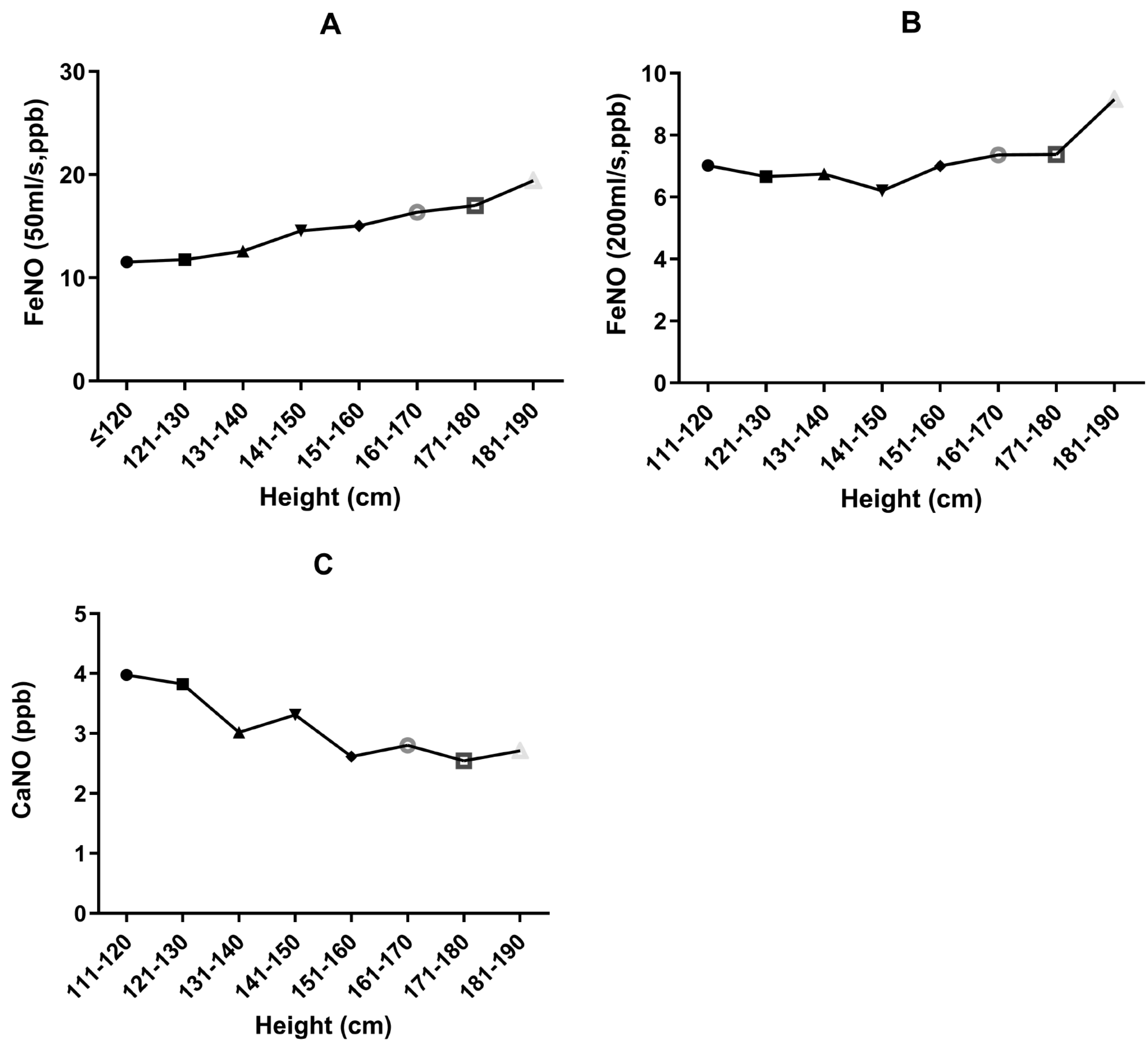

Fig. 6 $FeNO_{50}$, $FeNO_{200}$ and CaNO values of children with different heights

low, which is consistent with the results of the 2007–2010 National Health and Nutrition Examination Survey of the United States, in which, no gender difference in $FeNO_{50}$ value was found in children aged 6–11 years old, and the $FeNO_{50}$ value of 12–80 years old also showed that males were higher than females [8].

BMI, as an important confounding factor of eNO, our study found that no significant correlation between eNO and BMI or obesity (e-Table 3). In the Third National Health and Nutrition Survey, there was a significant positive correlation between BMI and asthma [34]. Some cross-sectional studies have also found that obesity is associated with asthma diagnosis, respiratory symptoms and airway hyperresponsiveness [35, 36]. Therefore, it is important to clarify whether BMI or obesity affects eNO. Our study found no significant correlation between eNO and BMI. According to the Chinese childhood obesity standard, the subjects were divided into obese group and non-obese group, and the effect of obesity on eNO was further analyzed. The results showed that the eNO did not differ between obese and non-obese children, similar to the previous study [37]. Nonetheless, we could not exclude the possibility that airway inflammation in obese subjects may be caused by immune mechanisms that are independent of NO.

Our study found that the value of $FeNO_{50}$ was significantly correlated with regions, which was significantly higher in Middle and Northwest China. The reasons may be related to China's vast surface area, environmental pollution, and regional differences in eating preferences.

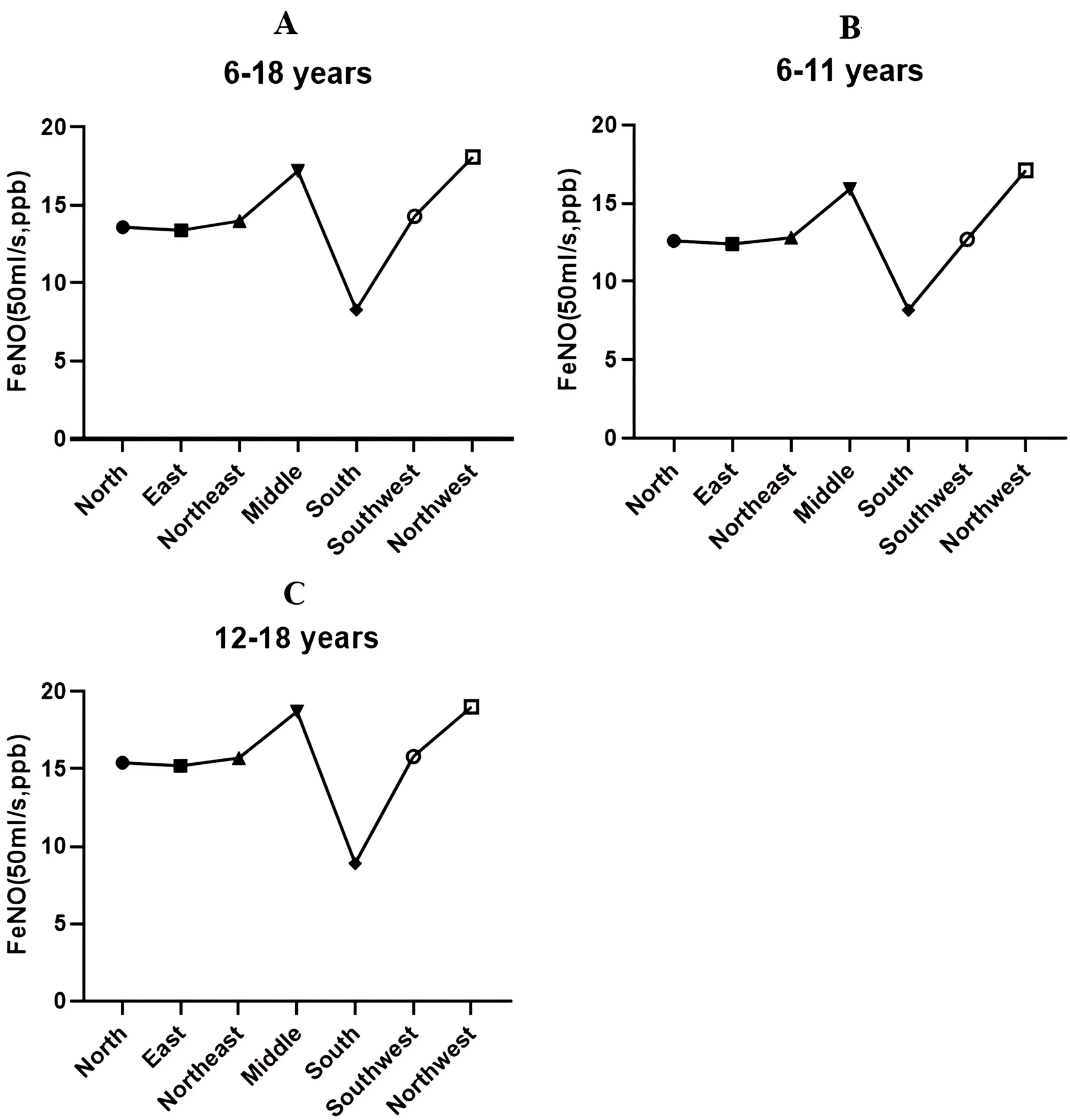

Fig. 7 $FeNO_{50}$ values of children with different regions

Environmental pollution has an important impact on the value of $FeNO_{50}$ [33]. Previous studies found that the $FeNO_{50}$ level of Asian people is more significantly correlated with PM2.5 [38]. The air pollution in southern China is better than in other areas, which may make the $FeNO_{50}$ value of children in southern China lower than in other areas. Diet also has a significant effect on the values of eNO. NO is produced both by oxygen-dependent NO synthases catalyzing its production from L-arginine and an alternative nitrate (NO_3^-)–nitrite (NO_2^-)–NO pathway [39]. The latter can be influenced by supplementation with exogenous dietary NO_3^-. Studies have shown that dietary nitrate supplementation, such as spinach, lettuce, broccoli, radish and so on, can effectively increase the concentration of nitrite and nitrate, and can increase the concentration of $FeNO_{50}$ [40, 41]. In contrast, the middle and northwestern regions of China were more prone to eating preserved food, which may also be one of the reasons for the increase in the value of $FeNO_{50}$.

Table 2 Regression coefficients (β) and *p*-values of the multiple regression models for eNO parameters in age group 6–11 years

	Interceptβ	Age		Gender		Height		BMI		Regions			
		β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	R^2	R^2boot
$FeNO_{50}$	1.718	0.054	< 0.001	-0.014	0.412	0.003	< 0.05	-0.001	0.958	0.026	0.116	0.036	0.036
$FeNO_{200}$	2.308	0.011	0.704	-0.061	0.259	-0.001	0.784	-0.014	0.122	-	-	0.013	0.001
CaNO	2.750	0.014	0.786	-0.199	< 0.05	-0.012	0.148	0.012	0.485	-	-	0.020	0.009

The R^2 is the unadjusted coefficient of determination of the models and R^2 boot is the corresponding optimism- corrected R2 values as estimated by bootstrapping

Table 3 Regression coefficients (β) and p-values of the multiple regression models for eNO parameters in age group 12–18 years

	Interceptβ	Age		Gender		Height		BMI		Regions			
		β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	R^2	R^2boot
$FeNO_{50}$	2.396	-0.010	0.638	-0.123	< 0.001	0.003	< 0.05	-0.026	0.195	0.023	< 0.001	0.023	0.022
$FeNO_{200}$	0.501	-0.115	0.052	-0.077	0.187	0.009	< 0.001	-0.082	0.142	-	-	0.043	0.040
CaNO	1.740	-0.050	< 0.05	-0.005	0.929	-0.008	0.896	0.038	0.514	-	-	0.017	0.014

The R^2 is the unadjusted coefficient of determination of the models and R^2 boot is the corresponding optimism- corrected R^2 values as estimated by bootstrapping

This study had several limitations. First, the healthy children in this study were defined through the questionnaire survey and the pulmonary function measurement, without the measurement of eosinophil count or total IgE level, which could not completely exclude children with allergic diseases. Second, $FeNO_{200}$ and CaNO tests have been performed on children from two centers in East China, which cannot fully reflect the level of children in the whole of China. Third, research indicates that latitude and longitude, which have a significant impact on temperature and environment, may also have an impact on the reference value of eNO due to the higher risk of asthma at low latitudes [42, 43]. Therefore, these issues should be considered in future studies.

Conclusion

In summary, we investigated the normal values and influencing factors of $FeNO_{50}$, $FeNO_{200}$ and CaNO in healthy children and adolescents aged 6–18 years in China. Age, height and gender, as important physiological indicators, were associated with $FeNO_{50}$, $FeNO_{200}$ and CaNO. The influence of regional differences on eNO was not only reflected in the differences among regions of China, but also in the differences in expiratory values between Chinese, North American and European children, further confirming that the eNO values of the Asian population are higher than those of European and American population. Although, there some several limitations in this study, it is still the largest study of normal eNO in healthy children so far, which can provide some recommendations for the clinical application of eNO in international children, particularly in Asian children.

Abbreviations

NO	Nitric oxide
eNO	Exhaled nitric oxide
FeNO	Fractional Exhaled Nitric Oxid
$FeNO_{50}$	Fractional concentration of exhaled nitric oxide at a 50 ml/s flow rat
$FeNO_{200}$	Fractional concentration of exhaled nitric oxide at a 200 ml/s flow rat
CaNO	Concentration of nitric oxide of the alveolar or acinar region
ATS	American Thoracic Society
ERS	European Respiratory Societ
GINA	Global Initiative for Asthma
NICE	National Institute for Health and Care Excellence
BMI	Body-Mass-Index
ICS	Inhaled Corticosteroids
PM	Particulate matter
IgE	Immunoglobulin E

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

Yazun Liu drafted the manuscript. Hao Zhang designed the study, and contributed to the manuscript revision of the manuscript draft. Acquisition, analysis, or interpretation of data: All authors (Yazun Liu, Hao Zhang, Jinrong Wang, Yuling Han, Chunhong Pan, Wenhui Jiang, Chunyan Ma, Yongsheng Shi, Chunmei Jia, Yuehua Zhang, Ming Li, Fei Wang, Yanyan Yu, Yong Feng, Li Liu, Aihong Liu, Qiaoling Zhang, Zhen Long, Fuli Dai, Yanli Zhang, Minghong Ji, Dongjun Ma). All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Shanghai Children's Medical Center (Ethics No: SCMCIRB-K2017007), and each subcenter follows the master research unit ethics. All participants from this study (Trial registration: Chinese Clinical Trial Registry ChiCTR 1800019029) signed an informed consent form.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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