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Reference Values of Exhaled Nitric Oxide in Healthy Asian Children Age 5 to 18 Years

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ABSTRACT

This study was undertaken to establish reference values of fraction of exhaled nitric oxide (FeNO) and its determinants in healthy Asian children.

Six hundred and ninety-three healthy Asian children aged 5-18 years were assessed using a single-breath online FeNO measurement (exhaled flow 50 mL/second), questionnaires, anthropometric measurements, spirometry, and total and specific immunoglobulin E (IgE).

Geometric mean FeNO and the upper 95% confidence limit were 13.7 ppb and 49.7 ppb, respectively, for healthy children and 11.2 ppb and 30.2 ppb, respectively, for those without allergic sensitization. FeNO was positively associated with age, allergic sensitization, total IgE, ambient nitric oxide, measurement in the afternoon, and drinking water within one hour before testing, and negatively associated with weight. In healthy children without allergic sensitization, age was the single best explanatory variable. The FeNO predicted values were 1-2 ppb higher in Asian than in Caucasian children in earlier studies, while the upper 95% confidence limits were 9-10 ppb higher.

In conclusion, the upper limits of normal FeNO in Asian children depend on age, from 21 ppb in young children to 39 ppb in adolescents. Ethnicity, age, allergic sensitization, total IgE, ambient nitric oxide, time of testing, drinking water, and weight are important determinants.

Key words: *Exhaled nitric oxide, reference values, prediction equations, children, Asian, age.*

Abbreviations: ATS, American Thoracic Society; CI, confidence interval; ERS, European Respiratory Society; FEF, forced expiratory flow; IgE, immunoglobulin E; FeNO, fraction of exhaled nitric oxide; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; ISAAC, International Study of Asthma and Allergies in Childhood; NO, nitric oxide; PATCH, Prediction of Allergies in Taiwanese Children; URI, upper respiratory infection; ppb, parts per billion; ULN, upper limit of normal.

INTRODUCTION

There is growing interest in the potential application of measurement of the fraction of nitric oxide (FeNO) in the diagnosis and management of asthma from research to clinical practice [1-5]. More work is required to determine reference values for appropriate interpretation [1, 2] and although there have been a few studies presenting reference values of FeNO for children, these have focused on Caucasian populations [6-8]. Current estimates indicate about 13.2 million people, or around 4.4%, of the entire United States population are of Asian descent [9]. Ethnic differences of FeNO levels between Caucasians and Asians have been reported in previous Western studies, as FeNO levels are significantly higher in Asian children [7, 8, 10]. Whether FeNO determinants in Asian children also differ from those reported in Caucasian children remains largely unknown.

Nonetheless, it is clear that reference values derived from Caucasian children cannot simply be applied to Asian children. Unfortunately, the small sample sizes of Asian sub-groups in previous studies do not provide reliable reference values for clinical application. Thus, there is an unmet need for reference values and determinants of FeNO in Asian children [8]. This study aimed to establish FeNO reference values and determinants in a large population-based sample of healthy Asian children based on current measurement standards.

METHODS

Subject recruitment

The study subjects were enrolled from the Prediction of Allergies in Taiwanese Children (PATCH) study, a population-based cohort study that was launched in 2007 to

investigate the epidemiology and predictive factors of asthma and allergies in children [11]. The subject flow diagram was presented in Fig. 1. Of the 1900 children invited to participate, 1717 agreed to participate, representing a participation rate of 90.4%. There was no significant difference in terms of age, gender, and prevalence of asthma, allergic rhinitis, and atopic dermatitis between these 1717 subjects and the 5351 children in the original cohort, indicating a sampling cohort representative of the general population. Parents of the 1717 subjects answered questionnaires regarding demographic data, general health information, and questions on clinical symptoms and diagnosis of allergic diseases. Healthy subjects were selected by including all children who had no chronic illnesses, no history of asthma, allergic rhinitis, or atopic dermatitis, and no current or past symptoms of wheeze, rhinitis, or eczema defined by the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [12]. Subjects who smoked or had missing answers on questionnaires were also excluded.

The remaining 693 healthy children (age range, 5-18 years) who were finally included in this study were all born to parents who were both of Asian descent (mostly Chinese). The Institutional Review Board of Chang Gung Medical Foundation approved the study (96-0370B) and the parents of each subject provided written informed consent.

Exhaled nitric oxide and pulmonary function

The FeNO was measured in all subjects by a single-breath online method using a chemiluminescence analyzer (CLD 88sp NO analyzer, Eco Medics, Duernten, Switzerland) according to the 2005 ATS/ERS guidelines [13]. All of the subjects were requested to avoid eating, drinking, and strenuous exercise one hour before the FeNO measurements. Ambient nitric oxide (NO) and temperature were recorded. A

representative tracing of FeNO measurement was shown in Fig. 2. Subjects inhaled NO-free air through a mouthpiece of the DENOX 88 NO-free air supply module over a period of 2-3 seconds to total lung capacity, minimizing contamination of ambient NO. Subjects then exhaled at a flow rate of 50 mL/second and against enough resistance to maintain an oro-pharyngeal pressure of 5-20 cm H_2O, thus preventing contamination of nasal NO. An exhalation time of 12 seconds was used as default, while in children younger than 12 years, exhalation time was reduced to 6 seconds if needed. Repeated exhalations were performed for a maximum of six attempts in order to obtain three acceptable plateau FeNO values that agree within 10% or 2 values within 5%. The mean FeNO was recorded.

After FeNO measurements, the subjects received spirometry (Spirolab II, Medical International Research, Roma, Italy) in accordance with the ATS/ERS recommendations [14] and percentages of the predicted values of pulmonary function variables (i.e., FVC, FEV_1, FEV_1/FVC ratio, and FEF_{25-75}) were calculated [15].

Total and allergen-specific serum IgE

The serum level of total immunoglobulin (IgE) was determined by ImmunoCAP (Phadia, Uppsala, Sweden). Specific IgE was determined by a commercial assay for IgE (ImmunoCAP Phadiatop Infant, Phadia, Uppsala, Sweden) against the most common inhalant and food allergens (i.e., house dust mite, cat, dog, birch, timothy, ragweed, wall pellitory, egg white, cow's milk, peanut, and shrimp). The cut-off values for each Phadiatop Infant class 0, 1, 2, 3, and >3 are 0, 0.35, 0.7, 3.5, and ≥17.5 kU/L, respectively. Values of Phadiatop Infant of 0.35 kU/L or more (≥ class 1) were considered indicative of allergic sensitization [16].

Statistical analysis

All data analyses were performed using the SPSS statistical package version 15.0 for Windows (SPSS, Chicago, IL). The FeNO values appeared to be log-normally distributed and were therefore logarithmically transformed for analysis. The results were presented as back-transformed values (i.e., geometric means and upper 95% confidence limits). Univariate analyses were performed using simple linear regression and Student *t* tests to assess associations between log-transformed FeNO and the following explanatory variables: age, anthropometric measurements, pulmonary function variables, total IgE, ambient NO and temperature, gender, allergic sensitization, symptoms of upper respiratory infection (URI) in the past two weeks, passive smoking, premature birth, time of testing (i.e., morning [9:00-12:00 AM] or afternoon [1:30-4:30 PM]), and drinking water within one hour before testing. Polynomial linear trend analysis was used to examine if there was a significant linear trend between increasing height of Phadiatop Infant class and FeNO.

For multivariate analyses, variables with $p<0.1$ in univariate analyses were included in the multiple linear regression model, while a forward stepwise selection with an entry probability of 0.05 and a removal probability of 0.1 from an *F*-test was used to select the final model. To evaluate the effectiveness of the fitted model in predicting the log-transformed FeNO, adjusted R^2 values as a measure of goodness-of-fit of the model was examined. Interactions between variables in the final model were tested and no significant interactions were identified. There was no collinearity in the models as the R matrix did not include r-values above our threshold of 0.8. Residual analysis with residual plots and normal probability plots of residuals confirmed no violation of the linear regression assumptions. A threshold of $p<0.05$ was considered statistically significant.

RESULTS

Subject characteristics

Acceptable FeNO measurements were available in 661 of 693 study subjects (95.4%; demographic data in Table 1). The relatively lower proportion of boys in this cohort was attributed to the exclusion of children with allergic diseases, which were more common in boys in the general population. Acceptable pulmonary function tests and total and specific IgE levels were available in 650 (98.3%) and 512 (77.5%) of subjects, respectively.

All healthy Asian children as a group

The geometric mean FeNO level in the entire healthy children population was 13.7 parts per billion (ppb) and the upper 95% confidence limit was 49.7 ppb. Six children (four boys) were outliers (defined by box plot outlier rules) for FeNO, predominantly those with allergic sensitization (all four received blood sampling). Two outliers reported recent symptoms of URI: one had allergic sensitization and the other drank water within an hour before testing. These outliers were unlikely to result from an error in data recording and were therefore not excluded from the analyses. However, the six outliers were not included in the calculation of FeNO reference values in healthy children without allergic sensitization because four of them had allergic sensitization and the other two refused blood sampling.

Univariate analyses (Table 2) showed that age, height, body surface area, several pulmonary function variables, total IgE, and ambient NO were significantly correlated with FeNO levels (all $p<0.05$). There were significant differences in FeNO levels among subjects grouped by allergic sensitization ($p<0.001$), time of testing ($p=0.006$), or drinking water within one hour before testing ($p=0.006$). Interestingly, there was a significant

positive correlation between FeNO level and increasing height of Phadiatop Infant class (polynomial linear trend analysis $p<0.001$; Fig. 3).

Multivariate analyses demonstrated that FeNO was positively associated with age, allergic sensitization, total IgE, ambient NO, measurement in the afternoon, and drinking water within one hour before testing, and negatively associated with weight (equation 1; Table 3). Altogether, these factors accounted for 21.7% of total variability.

Healthy Asian children without allergic sensitization

The status of allergic sensitization was taken into account in 512 of 661 subjects who received blood sampling. The geometric mean FeNO and the upper 95% confidence limit in the sub-group of 213 healthy subjects with allergic sensitization were 17.7 ppb and 74.8 ppb, respectively. In contrast, the geometric mean FeNO and the upper 95% confidence limit in 299 healthy subjects without allergic sensitization were 11.2 ppb and 30.2 ppb, respectively. In the stepwise regression analysis, age appeared to be the best independent variable explaining variability of FeNO. After introducing age into the model and adjusting for other variables, no other factors had significant and independent effects on FeNO. Thus, the regression equation finally selected for healthy subjects without allergic sensitization was a simple model that had age as the single best explanatory variable: Ln FeNO= 1.933 + age [yr] × 0.046 (equation 2; Table 3).

In order to facilitate comparability of the results to those reported in Caucasian children, another regression equation was presented by further excluding 79 subjects who reported symptoms of URI in the past two weeks (n=220): Ln FeNO= 1.892 + age [yr] x 0.048 (equation 3; Table 3). The predicted FeNO values and the upper 95% confidence limits based on equation 3 were presented in Fig. 4. For comparison, the results were

shown with data in a sample of healthy non-atopic Caucasian children [6]. Compared to recent data of Caucasian children [6, 7], the predicted FeNO values were approximately 1-2 ppb higher in healthy children without allergic sensitization in the study population, and the upper 95% confidence limits were approximately 9-10 ppb higher in the current study.

DISCUSSION

This is the largest study to date of FeNO in healthy Asian children that defines the upper limit of normal (ULN; defined as the upper 95% confidence limit in accordance with earlier studies [6, 7]) for FeNO, which is age dependent. The upper limit ranges from 21 ppb at age 5 years to 39 ppb at age 18. Importantly, this study identifies that the predicted FeNO values in Asian children in the current population are approximately 1-2 ppb higher than in Caucasian children in earlier studies [6, 7] and the ULNs are approximately 9-10 ppb higher in the current study. Malmberg et al. [6] have established a regression equation for FeNO from 114 non-atopic non-smoking healthy Caucasian children aged 6.9-15.7 years in Finland. Therefore, direct comparison with the reference equation from the present study is possible. The ULNs for FeNO in Asian children in this study are approximately 9 ppb higher than in Caucasian children in the study by Malmberg et al. [6]. Buchvald et al. [7] have established reference values of FeNO from 405 healthy children aged 4-17 years (mostly Caucasians) in Europe and the United States. The ULN for FeNO ranges from 15 to 25 ppb depending on age and self-reported atopy [7], with an approximate 10-ppb difference compared to the corresponding values in the current study. Buchvald et al. [7] also note significantly higher FeNO levels in non-Caucasian children (Asians, African

Americans, and Hispanics). Consistent with the current study, FeNO levels in a relatively small group of Japanese children aged 10-12 years who are non-atopic and never experienced wheeze [17] are also higher than those of Caucasian children [6-8]. However, a recent study reports that FeNO reference values in healthy Japanese adults [18] are similar to those of Caucasians [19-21]. Thus, whether or not ethnic differences of FeNO levels between Caucasians and Asians are restricted to children warrants further study.

Explanations for the observed higher levels and particularly higher variation of FeNO in Asian children in the current study than in Caucasian children in previous studies must still be identified. It is important to recognize that only a small proportion of the variability of FeNO values can be explained here and in prior population-based studies [6, 21, 22] by determinants of FeNO identified so far, leading many to question how the remaining "missing variability" of FeNO can be explained. Interestingly, a population-based study of twins [23] suggests that genetic effects account for most of the FeNO variations, and that environmental factors have a minor role. Moreover, variants in the NO synthesis pathway genes contribute to differences in FeNO levels in children in a recent population-based study [24], supporting the former observation. Therefore, it is likely that genetic factors may account for the "missing variability" of FeNO. Given that inducible NO synthase (iNOS) in squamous and respiratory epithelia produces the vast majority of the NO detected in exhaled breath in normal subjects [25], it can be speculated that ethnic differences in the genetic regulation of NO synthase pathway may explain the differences of FeNO levels between Asian and Caucasian children. In addition, the observation that Asian children in the current population and a previous study [26] have relatively low spirometry values has led to speculation regarding differences in lung function to explain

FeNO differences between Asian and Caucasian children. This requires further investigation. There are also other possible explanations, including methodological factors, measurement conditions, heterogeneity of population, and less well-recognized environmental factors like air pollution, especially particulate matter and ozone, type of diet, differences in oral bacterial flora, and upcoming respiratory infections.

This large study demonstrates that FeNO is positively associated with age, allergic sensitization, total IgE, ambient NO, measurements in the afternoon, and drinking water, and negatively associated with weight. Thus, factors affecting FeNO in Asian children are generally similar to those reported in Caucasian children [6-8, 10, 13]. Although the mechanism for the age dependence of FeNO is largely unknown, having the same fixed expiratory flow in all ages is a possible reason [4]. With increasing height accompanying aging, the total airway mucosal surface area available for NO diffusion will increase which will lead to higher FeNO levels using the same exhalation flow rate [27, 28]. The age-dependence of FeNO has been suggested in previous smaller studies in children [7, 29-31].

Notably, allergic sensitization (defined as a value of Phadiatop Infant of ≥0.35 kU/L) is an important determinant of FeNO even in healthy children who have no history of allergic diseases and have no current or past symptoms suggestive of allergic diseases. This finding not only confirms the previous reported association of allergic sensitization with FeNO [6, 17, 29, 32], but further indicates that the association is quantitative and independent of symptoms. As such, a hypothesis is proposed here that FeNO elevation may denote a constitutional feature of allergic sensitization. Because recent evidence suggests that elevated FeNO in subjects without respiratory symptoms indicates a risk for

developing asthma-like symptoms and even clinical asthma [33, 34], longitudinal follow-up of healthy children with increased FeNO is warranted.

Even though this cross-sectional study with one measurement during the daytime is not ideal for investigating the circadian rhythm of FeNO, there is a significant trend of increased FeNO in the afternoon compared to the morning. This circadian FeNO variation in healthy children is consistent with previous findings in healthy adults [35, 36]. However, other studies demonstrate different or no circadian FeNO patterns [30, 37]. It has been established that there is increased FeNO after ingesting nitrate or nitrate-containing foods [38, 39], while mouthwash procedures immediately before the measurement reduce such influence [38]. Thus the rise in FeNO during the day may be attributed to dietary nitrate. However, given that the influence of dietary nitrate on FeNO reaches a maximum effect two hours after ingestion [38], it can be argued that refraining from eating for one hour prior to test, which is according to the current ATS/ERS guidelines [13], may be inadequate. Taken together, it is ideal to measure FeNO at the same time during the day, always question recent food intake, and perhaps add a mouthwash procedure immediately before measurements, particularly for longitudinal comparisons.

This study also demonstrates a small but significant negative association of weight with FeNO levels in healthy Asian children, estimated at 0.7% decrease per kg. A similar finding of increasing weight-for-height associated with decreasing FeNO is observed in a study of offline FeNO measurement in Southern California children [10]. A preliminary consensus reached between this study and that by Linn et al. [10] is that the influence of weight on FeNO levels is relatively small. Moreover, although ambient NO is generally low in this study and inhalation of NO-free air is applied to all measurements, ambient NO

still has a slight but significant influence on FeNO levels. It is therefore preferable, regardless of technique used, that ambient NO at the time of testing should be recorded and considered when interpreting FNO levels in children. Avoiding measuring FeNO within one hour after drinking fluids is recommended because the data here indicates that such behaviour significantly and independently affects FeNO.

Taken together, the reference values and determinants of FeNO in healthy Asian children established by the current study are particularly important for interpreting FeNO in children of Asian descent. The strength of this study stems from a large sample size, a wide age range, incorporation of spirometry and objective markers of atopy, application of current standards for measurement, and a thorough analysis. However, it should be noted that extrapolation of the results to Asian children living in other countries and cultures still needs further confirmation.

In conclusion, the upper limits of normal for FeNO in healthy Asian children depend on age, ranging from 21 ppb in young children to 39 ppb in adolescents. These are approximately 9-10 ppb higher than those measured in Caucasian children in earlier studies. Both host factors (e.g., ethnicity, age, allergic sensitization, total IgE, weight, and drinking water) and non-host factors (e.g., ambient nitric oxide and time of testing) are important determinants of FeNO in children.

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Table 1. Characteristics of study subjects

	All (n=661)	5-7 y (n=150)	8-10 y (n=231)	11-13 y (n=204)	14-17 y (n=76)
Continuous variable					
Anthropometric measurement					
Height (cm)	138.2 ± 14.7	121.7 ± 6.5	133.0 ± 6.9	147.5 ± 8.5	161.6 ± 6.8
Weight (kg)	36.1 ± 13.0	25.0 ± 6.1	31.5 ±7.4	42.8 ± 11.9	54.1 ± 10.2
Body mass index (kg/m^2)	18.3 ± 3.5	16.7 ± 2.8	17.6 ± 2.8	19.4 ± 3.9	20.7 ± 3.7
Body surface area (m^2)	1.17 ± 0.26	0.91 ± 0.13	1.07 ± 0.14	1.32 ± 0.21	1.55 ± 0.16
Pulmonary function					
FVC (L)	2.03 ± 0.65	1.44 ± 0.27	1.81 ± 0.33	2.32 ± 0.48	3.06 ± 0.63
FEV_1 (L)	1.77 ± 0.55	1.26 ± 0.22	1.57 ± 0.26	2.03 ± 0.39	2.68 ± 0.52
FEV_1/FVC ratio (%)	87.7 ± 6.0	87.4 ± 6.6	87.3 ± 5.7	88.3 ± 5.8	88.0 ± 5.9
FEF_{25-75} (L/s)	2.20 ± 0.74	1.60 ± 0.41	1.95 ± 0.42	2.54 ± 0.59	3.27 ± 0.74
FVC % predicted (%)	90.5 ± 11.3	94.6 ± 11.9	90.1 ± 10.1	89.0 ± 11.6	87.6 ± 10.9
FEV_1 % predicted (%)	89.9 ± 10.3	91.8 ± 11.0	89.7 ± 9.6	89.6 ± 10.7	87.2 ± 9.7
FEV_1/FVC % predicted (%)	97.7 ± 6.7	96.2 ± 7.1	96.9 ± 6.2	99.2 ± 6.6	99.3 ± 6.7
FEF_{25-75} % predicted (%)	90.7 ± 19.5	86.8 ± 20.8	91.0 ± 18.4	93.7 ± 19.8	89.4 ± 18.3
Total IgE (kU/L)	166.1 ± 391.1	144.1 ± 231.4	189.0 ± 523.4	138.0 ± 272.6	222.3 ± 468.6
Ambient nitric oxide (ppb)	2.51 ± 2.86	2.41 ± 2.46	2.19 ± 2.05	3.03 ± 3.84	2.31 ± 2.46
Ambient temperature (□)	23.0 ± 5.9	23.0 ±5.6	24.0 ± 5.8	23.7 ± 5.4	18.5 ± 6.1
Categorical variable					
Gender (male; %)	39.5	44.7	38.5	40.7	28.9
Allergic sensitization (%)*	41.6	48.3	40.9	39.8	35.6
Recent URI symptoms (%)†	29.4	34.0	27.1	26.3	35.2
Passive smoking (%)	53.2	52.4	51.1	54.9	57.1
Premature birth (%)	5.2	4.1	6.8	5.6	1.4
Time of testing (afternoon; %)	48.3	42.0	52.4	45.6	55.3
Drinking (%)‡	2.4	2.7	0.4	2.9	6.6

*Allergic sensitization was defined as a value of Phadiatop Infant of ≥0.35 kU/L.

†Recent URI symptoms refer to symptoms of upper respiratory infection in past two weeks.

‡Drinking refers to drinking water within one hour before testing.

Table 2. Characteristics and factors associated with FeNO in healthy Asian children, by univariate analyses

Continuous variable	**Mean ± SD**	**n**	**r**	***p* value**
Age (yr)	10.2 ± 2.6	661	0.176	**<0.001**
Anthropometric measurement				
Height (cm)	138.21 ± 14.7	661	0.136	**0.001**
Weight (kg)	36.1 ± 13.0	661	0.073	0.062
Body mass index (kg/m^2)	18.3 ± 3.5	661	-0.015	0.699
Body surface area (m^2)	1.17 ± 0.26	661	0.092	**0.018**
Pulmonary function				
FVC (L)	2.03 ± 0.65	650	0.102	**0.009**
FEV_1 (L)	1.77 ± 0.55	650	0.119	**0.002**
FEV_1/FVC ratio (%)	87.7 ± 6.0	650	0.073	0.064
FEF_{25-75} (L/s)	2.20 ± 0.74	650	0.125	**0.001**
FVC % predicted (%)	90.5 ± 11.3	650	-0.104	**0.008**
FEV_1 % predicted (%)	89.9 ± 10.3	650	-0.065	0.096
FEV_1/FVC % predicted (%)	97.7 ± 6.7	650	0.091	**0.020**
FEF_{25-75} % predicted (%)	90.7 ± 19.5	650	0.010	0.796
Total IgE (kU/L)	166.1 ± 391.1	512	0.299	**<0.001**
Ambient nitric oxide (ppb)	2.51 ± 2.86	661	0.087	**0.026**
Ambient temperature (□)	23.0 ± 5.9	661	-0.009	0.822

			FeNO (ppb), Geometric mean (95% CI)		
Categorical variable	**%**	**n**	**yes**	**no**	***p* value**
Gender (male)	39.5%	261/661	13.5 (12.4─14.6)	13.9 (13.0─14.8)	0.567
Allergic sensitization*	41.6%	213/512	17.7 (16.0─19.5)	11.2 (10.6─11.9)	**<0.001**
Recent URI symptoms†	29.4%	187/637	14.3 (12.9─15.7)	13.5 (12.7─14.3)	0.347
Passive smoking	53.2%	339/637	14.1 (13.2─15.1)	13.3 (12.3─14.4)	0.234
Premature birth	5.2%	33/634	14.0 (11.3─17.3)	13.7 (13.0─14.5)	0.853
Time of testing (afternoon)	48.3%	319/661	14.7 (13.7—15.8)	12.8 (11.9—13.8)	**0.006**
Drinking‡	2.4%	16/661	21.4 (14.1─32.3)	13.5 (12.9─14.2)	**0.006**

*Allergic sensitization was defined as a value of Phadiatop Infant of ≥0.35 kU/L.

†Recent URI symptoms refer to symptoms of upper respiratory infection in past two weeks.

‡Drinking refers to drinking water within one hour before testing.

Table 3. Multiple linear regression models with Ln FeNO as the dependent variable in healthy Asian children*

	Equation 1		Equation 2		Equation 3	
	All healthy subjects (n=661)		Subjects without allergic sensitization (n=299)		Subjects without allergic sensitization and recent URI symptoms (n = 220)	
Variable	**Coefficient (95% CI)**	***p* value**	**Coefficient (95% CI)**	***p* value**	**Coefficient (95% CI)**	***p* value**
Intercept	1.536 (1.277–1.796)	<0.001	1.933 (1.697–2.170)	<0.001	1.892 (1.616–2.169)	<0.001
Age	0.061 (0.031–0.092)	<0.001	0.046 (0.024–0.068)	<0.001	0.048 (0.022–0.074)	<0.001
Allergic sensitization*	0.299 (0.162–0.435)	<0.001	–	–	–	–
Ln Total IgE	0.107 (0.061–0.153)	<0.001	–	–	–	–
Ambient nitric oxide	0.027 (0.008–0.045)	0.005	–	–	–	–
Time of testing (afternoon) †	0.137 (0.022–0.253)	0.020	–	–	–	–
Weight	-0.007 (-0.012–-0.001)	0.024	–	–	–	–
Drinking‡	0.348 (0.017–0.679)	0.040	–	–	–	–

*Allergic sensitization was defined as a value of Phadiatop Infant of ≥0.35 kU/L.

†Compared to morning.

‡Drinking refers to drinking water within one hour before testing.

§Adjusted R^2 = 0.217 (equation 1), 0.051 (equation 2) and 0.052 (equation 3), respectively. Residual standard deviation = 0.578 (equation 1), 0.493 (equation 2) and 0.468 (equation 3), respectively.

FIGURE LEGEND

Figure 1. Schematic presentation of the recruitment process of the study subjects.

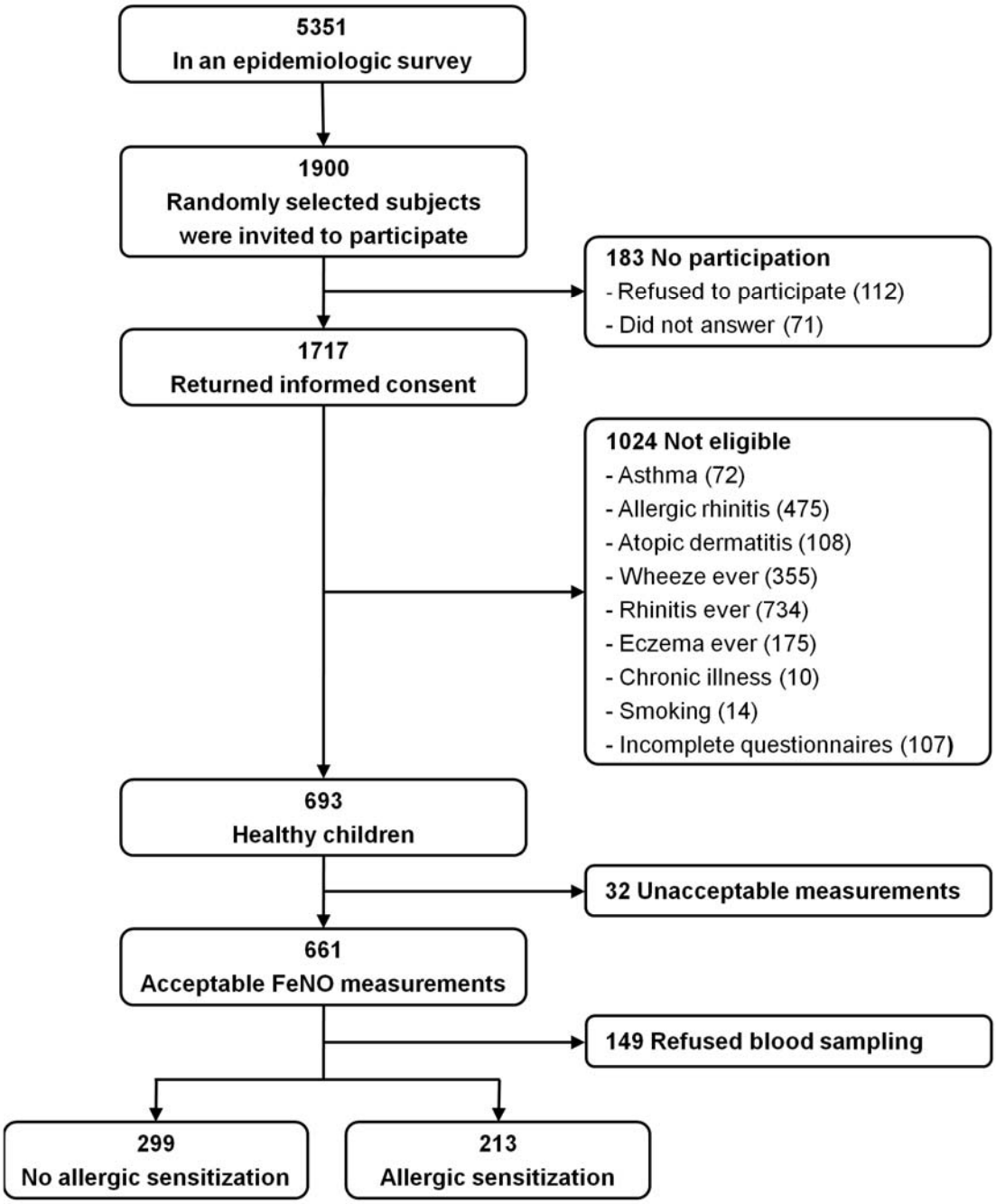

Figure 2. Representative tracing of exhaled nitric oxide (NO) measurement by a single-breath online method. Exhaled flow rate was 50 mL/second. An exhalation time of 12 seconds was used as default. Fraction of exhaled nitric oxide (FeNO), in parts per billion (ppb), was calculated during the 7-10 seconds (arrow) of exhalation.

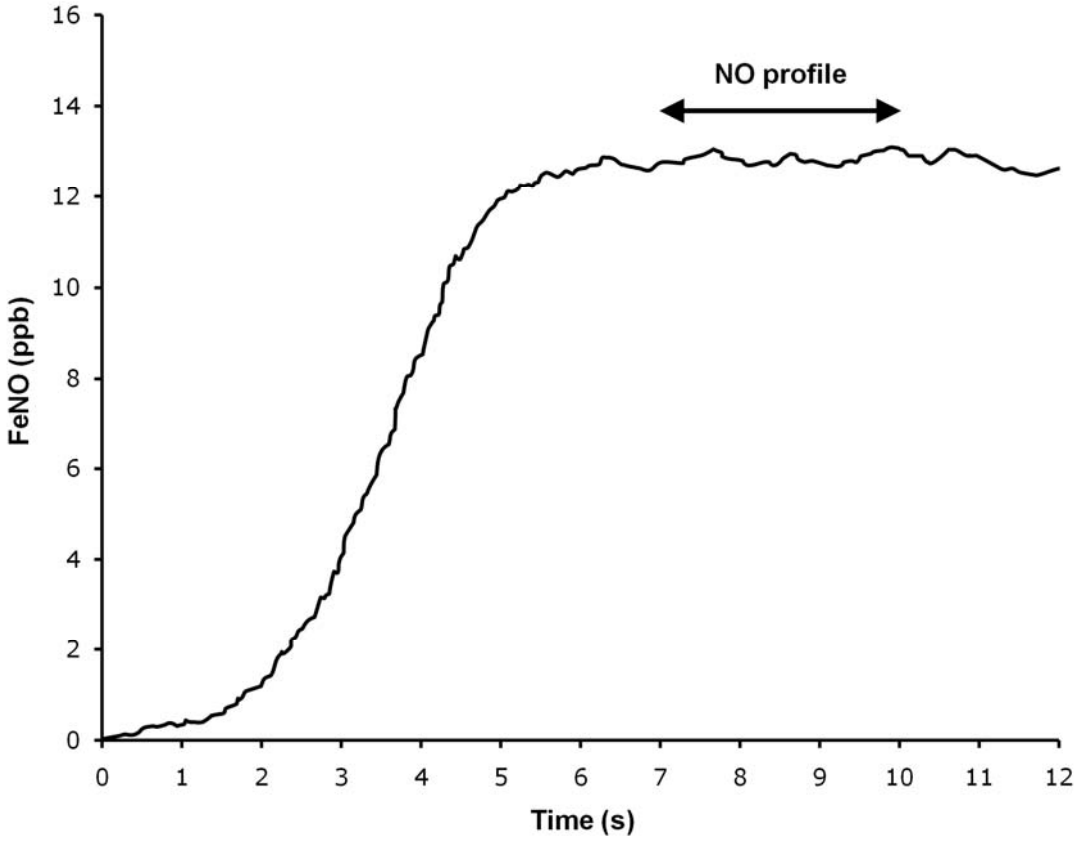

Figure 3. Box plots showing median and interquartile ranges of fraction of exhaled nitric oxide (FeNO) by Phadiatop Infant classes. The cut-off values for each Phadiatop Infant class 0, 1, 2, 3, and >3 were 0, 0.35, 0.7, 3.5, and ≥17.5 kU/L, respectively. Dots beyond the bounds of the whiskers denoted outliers. An increasing height of Phadiatop Infant class was significantly associated with FeNO levels by polynomial linear trend analysis (p<0.001).

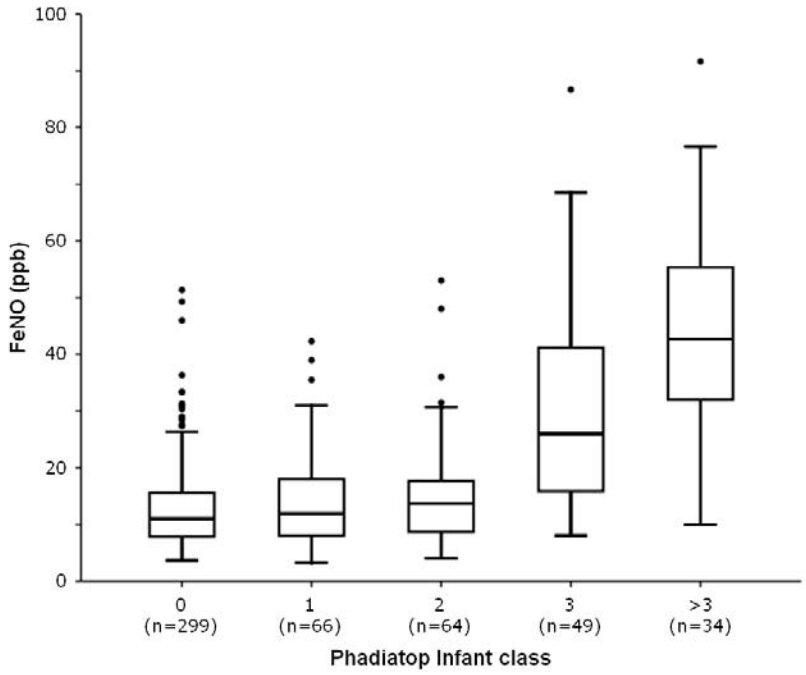

Figure 4. Predicted FeNO values (thick solid line) and upper 95% confidence limits (thick dashed line) were plotted as a function of age. Data from Malmberg et al. [6] (thin solid and dashed lines) in a sample of healthy non-atopic Caucasian children were included for comparison.