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Exhaled nitric oxide in healthy young children during tidal breathing through a facemask

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The aim of this study was to establish reference values and to examine day-to-day and within-day variations of exhaled nitric oxide (eNO) during tidal breathing in healthy children using a newly described method. Exhaled NO was measured on-line and off-line during tidal breathing through a facemask. In a subgroup of children measurements were repeated during the course of a single day and on the same time on three consecutive days. A total of 133 healthy children were included in the study and measurements were obtained from 121 children aged 2–7 yr (61 boys and 60 girls). The geometric mean eNO concentration and 95% CI was 3.9 (3.5–4.2) parts per billion (p.p.b.) for on-line measurements and 3.0 (2.7–3.3) p.p.b. for off-line measurements. Exhaled NO was independent of gender, age, height and weight. The 95% reference intervals (RI) for on-line and off-line measurements were 1.2–8.2 and 1.3–7.1 p.p.b. respectively. Twenty-three children completed measurements of within-day and day-to-day variations, none of which showed significant variation. In conclusion, the established reference values and data on variability within and between days may facilitate the clinical application for measurement of eNO during tidal breathing in young children.

Peter F. Daniel, Bent Klug and Niels H. Valerius
Department of Pediatrics, Copenhagen University Hospital, Hvidovre, Denmark

Key words: children; exhaled nitric oxide; facemask; on-line; off-line; tidal breathing; reference values; reference intervals; day-to-day variation; within-day variation

Correspondence
Peter F. Daniel, Department of Pediatrics, Copenhagen University Hospital, Hvidovre, Denmark
Tel.: 0045 363 22531
Fax: 0045 363 23770
E-mail: fleng@dadlnet.dk

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Exhaled nitric oxide (eNO) is a non-invasive marker of airway inflammation which may be useful for diagnosing and monitoring airway inflammation as in asthma (1–4). Measurements of eNO are performed as either on-line or off-line measurements, where the on-line measurements are performed simultaneously with the breathing manoeuvre whereas in off-line measurements eNO is collected in reservoirs for subsequent analysis.

Recommendations for standardized procedures for measurement of eNO have been outlined and recently revised (5, 6). However, methods that comply with these recommendations require considerable collaboration on the part of the patient and they are therefore only applicable in adults (7) and older children (8). Thus, children younger than 5 yr of age can rarely meet the demands set by the recommendations and there have been few reports on methods applicable in young children (9–11).

We have recently described a method for the measurement of eNO in young children during tidal breathing (12). In the present study, we applied this method to establish reference values in healthy children 2–7 yr old. Furthermore, we addressed the question of day-to-day and within-day variations.

Materials and methods

Subjects

Eleven kindergartens in the vicinity of Hvidovre Hospital were invited to join the study. Eight institutions gave their consent to participate. The study took place from March to May.

One week before the study was to take place written information containing information on aims and methods of the study and a health questionnaire was handed out to the parents.

Completed questionnaires and written consent were obtained from the parents of 243 children. All children were offered to participate but only data from those children who from the health questionnaire fulfilled the inclusion criteria (Caucasian, gestational age at birth above week 36, no neonatal airway disease, no family history of allergy, no former or present asthma, bronchitis or other chronic airway disease, no upper respiratory tract infection at present or within the past 4 wk, no medication and exposure to passive smoking less than five cigarettes/day) were included in this study.

Within-day and day-to-day variations were measured in a subgroup of children who had the opportunity to participate in the consecutive measurements. On the day of examination the equipment was brought to the kindergarten.

The study was approved by The Scientific Ethical Committee for the Municipalities of Copenhagen and Frederiksberg (KF 01-325/98).

Methods

The equipment and measurement procedures have been described in detail previously (12). In short, NO was measured on the Exhalyzer system (ECO Physics, Dürnten, Switzerland). In this setup the eNO was measured with a CLD AM 77 chemiluminescence analyser (ECO Physics), pressure with a Spiroson SCIENTIFIC ultrasonic flowhead (ndd Medizintechnik AG, Zurich, Switzerland) and NO-free air was supplied as scrubbed ambient air contained in a 30 l collapsible bag (Hans Rudolph Inc., Kansas City, MO, USA).

During inhalation NO-free air was led through a one-way valve on to a parted facemask (Hans Rudolph Inc.) which separated oral and nasal airflow. The nasal compartment of the facemask was fitted with a one-way valve to allow the pressure of the nasopharynx to equilibrate with the ambient pressure. Combined with an expiratory resistance this resulted in a mouth pressure above 4–5 cmH_2O thus securing a closure of the soft palate and a reduction of the admixture of nasally derived NO in the oral exhalant. Upon exhalation the exhalant was led through another one-way valve and the expiratory resistance into collapsible gas sampling bags (ADTECH; Stroud, Gloucester, UK) for subsequent off-line NO measurement. On-line eNO sampling and pressure measurement took place close to the facemask. Three eNO measurements were performed on each subject and the median measurement was used for further calculations. A custom-made software program was used for calculating the on-line eNO measurements (Riis-edb, Nærum, Denmark).

Day-to-day eNO measurements were performed on three consecutive days at the same time of the day ±1 h. Within-day eNO measurements were performed on the same day at 8 AM, 2 PM and 8 PM ±30 min.

Statistical analysis

After log-transformation of data eNO showed a log-normal distribution.

A two-sample t-test was used to compare values of eNO in boys and girls. Multiple linear regression was used to assess the influence of gender, age, height and weight on eNO.

Day-to-day and within-day variations were assessed with two-way analysis of variance. Statistical analysis was performed on a computer package (SAS Institute Inc., Cary, NC, USA); p-values < 0.05 were considered significant.

Results

A completed questionnaire and written consent was received from the parents of 243 children of whom 133 fulfilled the inclusion criteria. Twelve children (9%) refrained from participation either at the start (nine children: two aged 2 yr, two aged 3 yr, four aged 4 yr and one aged 5 yr) or during (three children 3, 4 and 6 yr of age) measurements. A total of 121 children participated in measurements for the reference values. Characteristics of these children are shown in Table 1 and Fig. 1. The geometric mean eNO (95% CI) for all children was 3.9 (3.5–4.2) parts per billion (p.p.b.) for on-line measurements and 3.0 (2.7–3.3) p.p.b. for off-line measurements. No significant difference was found between boys [on-line 3.8 p.p.b. (3.4–4.3) p.p.b., off-line 2.7 (2.4–3.2) p.p.b.] and girls [on-line 3.9 (3.5–4.3) p.p.b., off-line 3.3 (2.9–3.7) p.p.b.]. Off-line measurements were significantly lower than their corresponding on-line measurements. The influence of gender and age on on-line eNO concentrations is shown in Fig. 2.

Table 1. Characteristics of 121 healthy children who completed measurements of exhaled nitric oxide

	n	Age, mean (SD) (yr)	Height, mean (SD) (cm)	Weight, mean (SD) (kg)
Boys	61	5.0 (1.0)	111.7 (7.5)	19.3 (2.8)
Girls	60	5.0 (1.1)	111.5 (6.6)	19.1 (2.4)

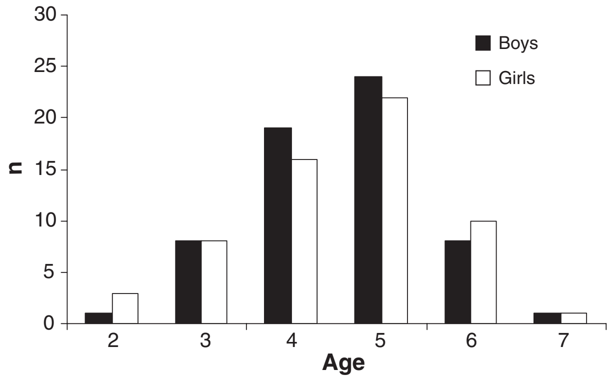

Fig. 1. Distribution on age and gender of 121 healthy children who completed measurements of exhaled nitric oxide.

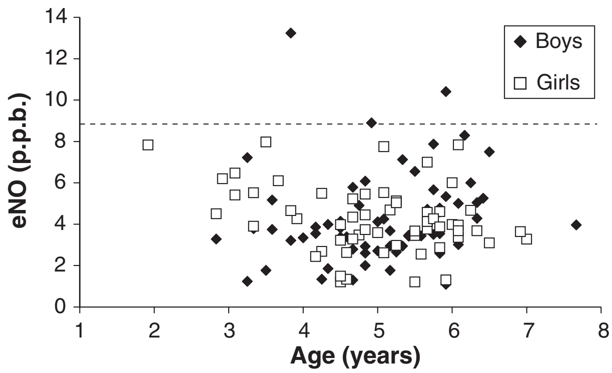

Fig. 2. Distribution of exhaled nitric oxide on gender and age in 121 healthy children; - - - - denotes upper 95% limit for the reference interval.

Multiple regression analysis showed no influence of gender, age, height or weight on eNO concentrations. For on-line and off-line methods 95% reference intervals (RI) were calculated as 1.2–8.2 and 1.3–7.1 p.p.b. respectively.

All 23 children, 2–7 yr old [mean (SD) 5.1 (1.1) yr], included in the studies of the day-to-day and within-day variability of eNO completed measurements. The differences in levels of eNO in both day-to-day and within-day variations were small and not significant. This was the case for both on-line and off-line measurements. Individual data for day-to-day and within-day eNO on-line measurements are shown in Fig. 3.

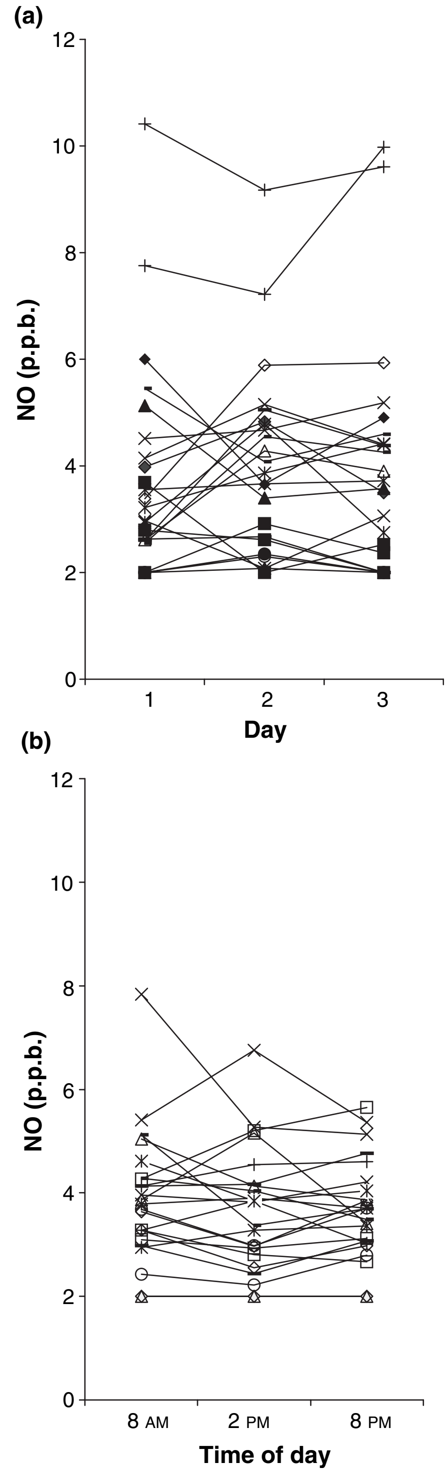

Fig. 3. Individial on-line measurements of exhaled nitric oxide in 23 healthy children. (a) Measurements performed on three consecutive days. (b) Measurements performed three times on a single day.

Discussion

In this study, we established reference values for eNO during tidal breathing in healthy young children using a recently described approach (12). Among 133 children previously unfamiliar with the method 121 completed the measurements, indicating that the method is well accepted by young children.

Some authors have found eNO levels in healthy children during tidal breathing comparable with those found in this study both on-line (10, 13) and off-line (14). Others have reported somewhat higher on-line (15, 16) and off-line (17, 18) values. However, a direct comparison of eNO values between laboratories is hampered by methodological differences.

At present, there is no standard method for measurement of eNO during tidal breathing. Baraldi et al. (16) measured eNO on single plateaus between the third and sixth minute of tidal breathing. Buchvald and Bisgaard (10) used a variable expiratory resistance to secure constant expiratory flow during tidal breathing and Hall et al. (19) have reported eNO values based on mathematical calculations of single plateaus.

Guidelines on procedures for measurement of eNO (5, 6) recommend exhalation at a fixed flow rate. However, young children are not able to sustain a fixed expiratory flow as recommended and the recommendations are therefore not applicable in young children unless some technique for controlling expiratory flow is applied (9–11, 20). In our experimental setup a fixed flow rate was not applied. Instead we used a custom-made software program to select and calculate eNO values from the short-lasting plateaus that occur during tidal breathing (12).

We did not find any influence of gender, age, height and weight on eNO. This is in accordance with some studies (16, 21, 22), while others have found a positive linear correlation between age and eNO (23–25). The lack of correlation in our study may be ascribed to the narrow age range of the study population.

Day-to-day and within-day variations showed no significant variation in levels of eNO neither for on-line nor for off-line measurements. There have been few reports on day-to-day and within-day variations and these have only included few subjects. In a group of 10 adults where eNO was measured 10 times during 14 days (26) no day-to-day variation was found. Similar findings were found in a group of six children measured on six consecutive days (25). Within-day variation was found in a group of six children demonstrating the highest eNO values in the evening (27) whereas no within-day variation was found in another group of six children (25).

In conclusion, the reference values established in this study should facilitate the clinical application of measurement of eNO during tidal breathing in young children.

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