Fraction$^{Q1}$ of Exhaled Nitric Oxide Norms ($\text{FeNO}$) in Healthy North African Children 5–16 Years Old

Sonia Rouatbi, MD, PhD,1,2* Ashraf Alqodwa, MD,1 Samia Ben Mdella, MD,1 and Helmi Ben Saad, MD, PhD1,2

Summary. Aims: (i) To identify factors that influence the $\text{FeNO}$ values in healthy North African, Arab children aged 6–16 years; (ii) to test the applicability and reliability of the previously published $\text{FeNO}$ norms; and (iii) if needed, to establish $\text{FeNO}$ norms in this population, and to prospectively assess its reliability. Population and Methods: This was a cross-sectional analytical study. A convenience sample of healthy Tunisian children, aged 6–16 years was recruited. First subjects have responded to two questionnaires, and then $\text{FeNO}$ levels were measured by an online method with electrochemical analyzer (Medisoft, Sorinnes [Dinant], Belgium). Anthropometric and spirometric data were collected. Simple and a multiple linear regressions were determined. The 95% confidence interval (95% CI) and upper limit of normal (ULN) were defined. Results: Two hundred eleven children (107 boys) were retained. Anthropometric data, gender, socioeconomic level, obesity or puberty status, and sports activity were not independent influencing variables. Total sample $\text{FeNO}$ data appeared to be influenced only by maximum mid expiratory flow (l sec$^{-1}$; $r^2 = 0.0236$, $P = 0.0516$). For boys, only 1st second forced expiratory volume (l) explains a slight ($r^2 = 0.0451$) but significant $\text{FeNO}$ variability ($P = 0.0281$). For girls, $\text{FeNO}$ was not significantly correlated with any children determined data. For North African/Arab children, $\text{FeNO}$ values were significantly lower than in other populations and the available published $\text{FeNO}$ norms did not reliably predict $\text{FeNO}$ in our population. The mean $\pm$ SD (95% CI ULN, minimum–maximum) of $\text{FeNO}$ (ppb) for the total sample was 5.0 $\pm$ 2.9 (5.4, 1.0–17.0). For North African, Arab children of any age, any $\text{FeNO}$ value greater than 17.0 ppb may be considered abnormal. Finally, in an additional group of children prospectively assessed, we found no child with a $\text{FeNO}$ higher than 17.0 ppb. Conclusion: Our $\text{FeNO}$ norms enrich the global repository of $\text{FeNO}$ norms the pediatrician can use to choose the most appropriate norms based on children’s location or ethnicity. Pediatr Pulmonol. 2012; 9999:1–15.

Key words: exhaled nitric oxide; norms; interpretation; child; Tunisia.

INTRODUCTION

Nitric oxide (NO) is produced by a wide variety of cell types including airway nerves, epithelial, inflammatory (macrophages, neutrophils, mast cells), and vascular endothelial cells. It is generated via a five-electron oxidation of a terminal guanidinium nitrogen on the amino acid L-arginine; this reaction is catalyzed by NO synthase.1–3 Since the demonstration of the presence of NO in the exhaled air by Gustafsson et al.4 in 1991, there have been several publications showing that the fraction of exhaled NO ($\text{FeNO}$) is elevated in many respiratory diseases, especially bronchial asthma in children.5,6 NO can be detected in exhaled air by several methods such as chemiluminescence, spectroscopy, electrochemical portable, and other methods currently under development.1,7 Cheaper and easy to use,8 $\text{FeNO}$ analyzers are now readily available and increasingly used not only for the diagnosis of eosinophilic airway inflammation Additional supporting information may be found in the online version of this article.

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Conflict of interest: None.

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Received 20 May 2012; Accepted 3 October 2012.
DOI 10.1002/ppul.22721
Published online in Wiley Online Library (wileyonlinelibrary.com).

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which is seen mainly in asthma,\(^9\) but also for its assessment.\(^{10,11}\) In addition, the American Thoracic Society and European Respiratory Society (ATS/ERS) have jointly demonstrated that some factors (i.e., age, atopy, and gender) may affect the FeNO values.\(^{12}\)

Interpretation of FeNO data relies upon comparison of measured values with predicted ones available from published norms (reference equations or normal values tables). However, to our knowledge, FeNO norms have only been established in four children populations.\(^{13–16}\) However, neither of these studies provided prospective verification for their studied populations, nor proposed a method of interpreting the measured FeNO (e.g., using an upper limit of normal (ULN) or a fixed percentage above which measured FeNO values would be considered as abnormal). The need for normal values specific to North African populations has been demonstrated for several physiological parameters.\(^{17–21}\) The applicability and the reliability of published FeNO norms\(^{13–16}\) should be assessed as regards to North African children, in order to avoid erroneous clinical interpretation of FeNO data in this population.

Moreover, the ATS/ERS have encouraged investigators to publish physiological norms for healthy populations of various racial backgrounds, to enable individual subject results to be compared with data from a racially similar population.\(^{12}\) The use of the same kind of assessment equipment and procedure is also recommended.\(^{12}\) Therefore, the present study aimed:

(1) To identify factors that influence the FeNO values of healthy North African, Arab children aged 6–16 years.

(2) To test the applicability and reliability of the previously published FeNO reference equations\(^{13–15}\) or normal values\(^{16}\) in this population (the null hypothesis is that there will be no difference between measured and predicted FeNO mean values).

(3) If needed, to establish FeNO norms in this population, and to prospectively assess its reliability.

**METHODS**

**Study Design**

This\(^{23}\) is a cross-sectional analytical study spread over 7 months (June 2011 to December 2011). It was conducted in the Department of Physiology and Functional Explorations in the Farhat HACHED Hospital in Sousse (Tunisia; altitude <100 m).

Study design consists of a sample of healthy Tunisian children (Arab race) in the region of Sousse, aged 6–16 years. Subjects were recruited from the children of the hospital workers, and from public and private schools. Information letters, clarifying the aims of the study, were put up in the Medicine Faculty and in the local different schools. When a child was interested, an appointment for medical questionnaires and exploration was fixed. Data from each volunteer child included: gender, age, height, weight, birth height and weight, smoking history (child or parents), medication use, medical history, physical examination, pubertal stage, sports activity, FeNO, and spirometry data. All children received a copy of their exploration, and when an unknown dysfunction was discovered, they were sent to a specialist. Study approval was obtained from the hospital Ethics committee, and written informed consent was obtained from all children and/or their parents.

**Sample Size**

The sample size is calculated according to the following predictive equation\(^{22}\): \(n = \frac{Z^2 \times p \times q}{\Delta^2}\), where “\(n\)” was the number of required children, “\(Z\)” was the 95% confidence level (\(= 1.96\)), “\(q\)” was equal to “\(1 - p\),” “\(\Delta\)” was the precision (\(= 7\%\)), and “\(p\)” was the estimation of children aged 5–16 years with a normal FeNO value. According to Buchvald et al.,\(^{16}\) among the 721 recruited children, only 405 children (\(P = 0.56\)) without outliers and atorics were retained (Supplementary E. Table 1). Plugging this relevant value into the predictive equation, the sample size was thus 193 children. Therefore, to determine the influencing factors and to establish FeNO norms, we recruited an initial group of 211 children (104 girls; 107 boys). To verify the reliability of our norms, we prospectively measured the FeNO in a second group of 24 additional healthy children (12 boys) meeting the inclusion criteria of the present study but having not participated to the first part.
Subjects

Volunteer children aged from 6 to 16 years were included. The following non-inclusion criteria were applied: chronic illnesses; a history of pulmonary diseases or related respiratory symptoms [history of asthma or medication asthma use, current or past symptoms of wheeze, chronic cough; abnormal lung function (obstructive ventilatory defect or abnormal spirometry data)]; oto-rhino-laryngologic diseases or symptoms [allergic rhinitis, rhinitis; symptoms and signs of acute upper respiratory infection during 2 weeks prior to assessment; recent upper airway infection (cold, flu, sore throat within the last 7 days)]; atopic dermatitis or eczema; regular medication use (especially steroids or β-agonist, leukotriene receptor agonist, etc.); heart disease; premature birth (i.e., birth before 36 weeks gestational age); active smoking; inability to perform properly FeNO measurement and imperfect realization of required respiratory maneuvers or inability to comply with the study procedure. The FeNO measure was performed after the non-inclusion criteria had been verified.

Medical Questionnaires

Two medical questionnaires recommended for epidemiological research were combined and used to assess several children characteristics. Questionnaires were written in Arabic and were composed of questions, mainly closed response and usually dichotomous. Questions were asked by an examiner with whom children or children-parents were not familiar. Children or parents of the subjects answered questionnaires regarding demographic data, general health information (especially birth weight, height, and gestational age noted from the personal health records), questions on clinical symptoms and diagnosis of allergic diseases.

Two subgroups of children were formed according to sports activity (non-active; active) based on the response to the following question: do you practice any sports activity outside of school? Two socioeconomic levels (SEL) were distinguished based on a socioeconomic score: ratio between the number of inhabited rooms and household size (<1.5: unfavorable; ≥1.5: favorable).

Physical Examination

Anthropometric data were verified, measured, or calculated: age, height, weight, body mass index (BMI), and body surface area (BSA, m²). The decimal age (accuracy to 0.1 years) was calculated from the date of measurement and the date of birth. Due to the failure of software to compute decimal age as the difference between test date and birth date, age was taken as the number of complete years from birth to the date of the study.

FeNO Measurement

The FeNO (expressed in parts per billion, ppb) was measured by Medisoft HypAir FeNO method using an electrochemical analyzer (Medisoft). The ATS/ERS recommendations were respected. The instrument was calibrated and used according to the manufacturer’s instructions, and work in conjunction with a personal computer. The software supplied by either manufacturer provided both audio and visual feedback allowing the participant to maintain a constant exhaled breath flow rate.

The online method, with constant flow rate, which is considered the method of choice, was used. The pediatric FeNO task force recommends expiratory flow rates of 50 ml/sec for online collection. After a full unforced exhalation outside the mouthpiece, a maximal inspiration was performed through an absorber to ensure NO-free air. The child then performed a controlled exhalation using flow control at an exhalation pressure of 4–10 cmH₂O for at least 6 sec, during which time sample collection and gas analysis was performed. Nasal contamination is presented by closure of the velum by using 5 cmH₂O oral back-pressure. A nose clip was not used. To encourage the child to exhale at a fixed flow rate of 50 ml/sec, the flow indicator on the device was replaced by a cartoon of a dolphin moving through hoops. Children were asked to not eat, drink, or participate in strenuous activity for 1 hr prior to the test.

Three acceptable measurements were taken at a flow rate of 50 ml/sec within a 15-min period according to ATS/ERS guidelines. The mean of the three values was used.

Spirometry Function Test

Spirometry was carried, according to the recent international recommendations, out in the sitting position,
and a nose clip was applied. All tests, performed by the same investigator using a spirometer (ZAN 100, Meßgeräte GmbH, Germany), were done after the FeNO measurement. The flow sensor of the spirometer was calibrated daily with a 3-L syringe.

The following parameters were measured/calculated: forced vital capacity (FVC, l); 1st second forced expiratory volume (FEV₁, l); forced expiratory flow from 25% to 75% of FVC (FEF₂₅–₇₅%, l sec⁻¹) and FEV₁/FVC ratio (absolute value). The results were compared with local age- and gender-matched reference values.

An obstructive ventilator defect was retained when the FEV₁/FVC ratio was lower than the lower limit of normal. FEV₁ and FVC were considered as abnormal when they were lower than the lower limit of normal.

Statistical Analysis

Data Analysis

For each child, the mean of the three FeNO values was used for statistical analysis. Preliminary descriptive analysis included frequencies for categorical variables and mean ± SD for continuous ones. The dependent variable (FeNO) was normally distributed and Fe NO measurement was calibrated with 1.64 times the residual standard deviation. The 95% CI (=1.64 × residual standard deviation) was calculated.

Comparison With Published Norms

Comparison was made by two ways:

(i) Individually measured FeNO were compared with the predicted FeNO from the published norms for the same age or height ranges as in the corresponding study, using paired t-tests and scatter plots or non-parametric tests and histograms. It is well known that FeNO values obtained with different devices are not directly comparable. As the Aerocrine devices are much more commonly used and most of the other devices give pretty similar results and as measurements on the HypAir FeNO are 1.6 times higher than those obtained with the Aerocrine NIOX and for a better interpretation of our data, we have adjusted our results in accordance with Brooks et al. For that reason all FeNO predicted values from the published norms were divided by 1.6 and individually measured FeNO were compared with the predicted/adjusted FeNO from the published norms as described above.

(ii) For more accuracy, a specific threshold (95% CI ULN) for each age range will be evaluated. For example, for a given child aged 12.0–12.9 years, FeNO value higher than the total sample FeNO 95% CI ULN, will be considered as abnormal.

Univariate and Multiple Regression Analysis: Influencing Factors

t-Tests were used to evaluate the associations between FeNO and the categorical variables (gender, SEL, sports activity, and obesity status). Pearson’s product-moment correlation coefficients (r) evaluated the associations between FeNO and the continuous measures (age, height, weight, birth height and weight, socioeconomic score, gestational age, BMI, BSA, qualitative score of Tanner, and pubertal stage by Tanner scale, FEV₁ (l, %), FVC (l, %), FEV₁/FVC (absolute value), FEF₂₅–₇₅% (l sec⁻¹, %)).

The linearity of association between FeNO and the continuous measures was checked graphically by plotting each regressor against the FeNO. Only significantly and linearly associated variables were entered into the model. A linear regression model was used to evaluate the independent variables explaining the variance in FeNO. Candidate variables were stepped into the model with a stepwise selection method. To determine entry and removal from the model, significance levels of 0.15 and 0.05 were used, respectively. No colinearity between predictors was detected with variance inflation factors. The linearity was evaluated by correlation (r) and determination (r²) coefficients and the standard error. The 95% CI (=1.64 × residual standard deviation) was calculated.

Pediatric Pulmonology
Reliability of the North African FeNO Norms

The reliability of our norms was evaluated in the second group of 24 healthy children. The number of children having a measured FeNO values higher than 95% CI ULN predicted normal values for each age range or for the total sample is determined. FeNO predicted normal values will be considered as reliable when no child from the second group will have a measured abnormal value.

Analyses were carried out using Statistica (Statistica Kernel version 6, StatSoft, France). Significance was set at the 0.05 level.

RESULTS

Children Data

An initial sample of 354 voluntary children Arab race was examined. Non-inclusion criteria, presented in detail in the Supplementary Data, were found in 119 children. Two hundred eleven healthy children (107 boys) were included to establish FeNO norms.

The SEL, sports activity, obesity, and puberty status of the 211 children are shown in Table 1. The two main conclusions from this table are: (i) Significantly fewer girls had a favorable SEL, or, were categorized as being active, than boys. (ii) Compared to boys, there are a significantly higher numbers of girls having a T5 of the qualitative score of Tanner or having level 3 of the pubertal stage by Tanner scale.

| TABLE 1—Socioeconomic Level, Sports Activity, Obesity, and Puberty Status |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | Boys (n = 107)  | Girls (n = 104) | Total sample (n = 211) |
| Socioeconomic level             |                 |                 |                  |
| Unfavorable                     | 85              | 95              | 180              |
| Favorable                       | 22              | 9               | 31               |
| Sports activity                 |                 |                 |                  |
| Active                          | 59              | 38              | 97               |
| Non-active                      | 48              | 66              | 114              |
| Obesity status                  |                 |                 |                  |
| Normal weight                   | 80              | 80              | 160              |
| Overweight or obesity           | 24              | 24              | 48               |
| Puberty status: qualitative     |                 |                 |                  |
| score of Tanner                 |                 | 5, 11, 16      |
| T1                              | 40              | 41              | 81               |
| T2                              | 26              | 25              | 51               |
| T3                              | 17              | 12              | 29               |
| T4                              | 19              | 15              | 34               |
| T5                              | 5               |                | 16               |
| Puberty status: pubertal        |                 |                 |                  |
| stage by Tanner                 |                 | 132             |
| 0                               | 66              | 66              | 132              |
| 1                               | 17              | 12              | 29               |
| 2                               | 19              | 15              | 34               |
| 3                               | 5               |                | 16               |

Comparison With Published Norms

Fraction of FeNO Healthy North African Children

The number of children in each age group, the gender distribution, the anthropometric, spirometric, and FeNO data are given in Table 2. Among included children, the birth weight, birth height, and FVC (% predicted) were significantly higher among boys than girls, and the former’s FEV1/FVC was significantly lower.

Supplementary E. Figure 1 shows the distribution of the 211 healthy children according to gender and age range. The two main conclusions from this figure are: (i) Compared to boys, there is a significantly lower number of girls aged 13.0–13.9 years. (ii) Compared to boys, there is a significantly higher number of girls aged 14.0–14.9 years.

The FeNO data of the 211 children are shown in Figure 1, according to age, height, and weight ranges (Fig. 1A–C, respectively). A significant FeNO difference is found between children at the age of 9.0–9.9 and 10.0–10.9 years.

Univariate Analysis

In the total sample, gender (Table 2), SEL, obesity status, and sports activity (Table 3) did not significantly affect the FeNO value.

The correlation coefficient (r) between FeNO and the continuous children’s data are shown in Table 3. For the total sample, FeNO was significantly correlated (P < 0.05) with FEF25–75% (l sec−1/C0). For boys, FeNO was significantly correlated (P < 0.05) with age and FEV1 (l). For girls, FeNO was not significantly correlated with any of the other variables.

Multivariate Analysis: FeNO Influencing Factors

Table 4 presents the cumulative r2 of the independent influencing factors included in the FeNO forward linear stepwise multiple regressions.

For the included boys, only FEV1 (l) explains a slight (r2 = 0.0451) but significant FeNO variability (FeNO (ppb) = 3.17682 + 0.75009 × FEV1 (l)). After the predicted FeNO value for a given boy was computed from this equation, the ULN for the child could be obtained by adding 4.8 ppb.

For the total sample, only FEF25–75% (l sec−1) explains a slight (r2 = 0.0236) but significant FeNO variability (FeNO (ppb) = 3.91283 + 0.46666 × FEF25–75% (l sec−1)). After the predicted FeNO value for an individual child was computed from this equation, the ULN for the child could be obtained by adding 4.6 ppb.

Comparison With Published Norms

Comparison Without Values Adjustment According to Brooks et al.33

Figure 2 shows individually measured FeNO plotted against the corresponding predicted value for the same
<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Boys (n = 30)</th>
<th>Girls (n = 24)</th>
<th>Boys (n = 27)</th>
<th>Girls (n = 21)</th>
<th>Boys (n = 32)</th>
<th>Girls (n = 32)</th>
<th>Boys (n = 107)</th>
<th>Girls (n = 104)</th>
<th>Sample (n = 211)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-6</td>
<td>6.5 ± 0.8</td>
<td>6.5 ± 0.9</td>
<td>6.6 ± 0.9</td>
<td>9.7 ± 0.9</td>
<td>12.5 ± 0.7</td>
<td>12.1 ± 0.7</td>
<td>15.2 ± 0.6</td>
<td>15.3 ± 0.9</td>
<td>105 ± 33</td>
</tr>
<tr>
<td>7-10</td>
<td>7.0 ± 1.0</td>
<td>7.1 ± 1.0</td>
<td>7.0 ± 1.0</td>
<td>10.2 ± 1.0</td>
<td>12.9 ± 1.0</td>
<td>11.9 ± 1.0</td>
<td>16.0 ± 1.0</td>
<td>16.1 ± 1.0</td>
<td>131 ± 35</td>
</tr>
<tr>
<td>11-13</td>
<td>8.5 ± 1.5</td>
<td>8.6 ± 1.5</td>
<td>8.5 ± 1.5</td>
<td>11.5 ± 1.5</td>
<td>13.5 ± 1.5</td>
<td>12.5 ± 1.5</td>
<td>17.5 ± 1.5</td>
<td>17.6 ± 1.5</td>
<td>162 ± 35</td>
</tr>
<tr>
<td>14-16</td>
<td>9.0 ± 2.0</td>
<td>9.1 ± 2.0</td>
<td>9.0 ± 2.0</td>
<td>12.0 ± 2.0</td>
<td>14.0 ± 2.0</td>
<td>13.0 ± 2.0</td>
<td>18.0 ± 2.0</td>
<td>18.1 ± 2.0</td>
<td>203 ± 35</td>
</tr>
</tbody>
</table>

*Data are mean ± SD.*

\*P < 0.05 (boys vs. girls) from one range to the next.

\*\*P < 0.05; boys versus girls.
Since age did not correlate with FeNO, we simply give a normal values range for FeNO for North African children aged 6–16 years. It is much simpler for clinicians to remember and device manufacturers to program.

The FeNO mean ± SD, 95% CI ULN and minimum–maximum of the 211 children are presented in Table 5. In practice, three ways can be used to interpret a measured FeNO value:

(i) Use of the total sample 95% CI ULN as a threshold. In this case, each child aged 5–16 years FeNO value higher than 5.4 ppb, is considered as abnormal.

(ii) For more accuracy, a specific threshold (95% CI ULN) for each age range is applied. For example, for a given child aged 12.0–12.9 years, each FeNO value higher than 7.7 ppb, is considered as abnormal.

(iii) Use of the total sample maximum FeNO value as a threshold. In this case, each child aged 5–16 years FeNO value higher than 17.0 ppb, is considered as abnormal.
Reliability of North-African FeNO Norms

The mean ± SD (95% CI) FeNO prospectively measured in the 24 children (10.6 ± 3.6 years, 141 ± 18 cm and 37 ± 14 kg) was 6.0 ± 3.0 ppb (4.8–7.3).

When we apply the normal values mentioned in Table 5, we found no child with a FeNO higher than the threshold (ULN) of 17.0 ppb or higher than the 95% CI ULN specific for each age range.

DISCUSSION

The FeNO of a large group of healthy North African/Arab children between 6 and 16 years old was prospectively measured. The available published FeNO norms did not reliably predict FeNO in our population and FeNO values are lower in healthy North African/Arab children than in other healthy populations. So, we can reject the null hypothesis that we would see no difference in the

### TABLE 3—Univariate Analysis Between the Fraction of Exhaled Nitric Oxide (FeNO) and Children’s Data

<table>
<thead>
<tr>
<th></th>
<th>Girls (n = 104)</th>
<th>Boys (n = 107)</th>
<th>Total sample (n = 211)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-standardized regression coefficient</td>
<td>Cumulative determination coefficient</td>
<td>P level</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>0.04</td>
<td>0.12</td>
<td>0.21</td>
</tr>
<tr>
<td>Height (m)</td>
<td>0.07</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.02</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td>-0.10</td>
<td>0.06</td>
<td>-0.01</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>0.02</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td>Birth weight (mg)</td>
<td>-0.04</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Birth height (kg)</td>
<td>-0.06</td>
<td>-0.03</td>
<td>-0.05</td>
</tr>
<tr>
<td>Duration of gestation (weeks)</td>
<td>-0.06</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Pubertal stage by Tanner scale</td>
<td>0.03</td>
<td>0.16</td>
<td>0.09</td>
</tr>
<tr>
<td>Qualitative scale of Tanner</td>
<td>0.01</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>Socioeconomic score</td>
<td>-0.12</td>
<td>-0.11</td>
<td>-0.11</td>
</tr>
<tr>
<td>Forced vital capacity (FVC) (l)</td>
<td>-0.01</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>-0.04</td>
<td>0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>1st second forced expiratory volume (FEV₁) (l)</td>
<td>0.03</td>
<td>0.21</td>
<td>0.12</td>
</tr>
<tr>
<td>FEV₁ (%)</td>
<td>-0.01</td>
<td>0.04</td>
<td>-0.02</td>
</tr>
<tr>
<td>FEV₁/FVC (absolute value)</td>
<td>0.06</td>
<td>-0.11</td>
<td>-0.01</td>
</tr>
<tr>
<td>Forced expiratory flow from 25 to 75% of FVC (FEF₂₅₋₇₅%; l sec⁻¹)</td>
<td>0.10</td>
<td>0.19</td>
<td>0.15</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅% (%)</td>
<td>0.14</td>
<td>0.02</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Univariate analysis between FeNO data and categorical variables

<table>
<thead>
<tr>
<th>Socioeconomic level</th>
<th>Unfavorable</th>
<th>Favorable</th>
<th>Sports activity</th>
<th>Active</th>
<th>Non-active</th>
<th>Obesity status</th>
<th>Normal weight</th>
<th>Overweight or obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls (n = 104)</td>
<td>4.9 ± 3.1</td>
<td>4.2 ± 1.7</td>
<td>5.4 ± 3.2</td>
<td>4.1 ± 2.1**</td>
<td>4.8 ± 3.0</td>
<td>4.7 ± 2.5</td>
<td>5.2 ± 3.0</td>
<td>5.1 ± 3.1</td>
</tr>
<tr>
<td>Boys (n = 107)</td>
<td>5.4 ± 2.9</td>
<td>4.4 ± 3.0</td>
<td>5.5 ± 2.7</td>
<td>5.1 ± 3.0</td>
<td>5.4 ± 3.0</td>
<td>4.8 ± 2.1</td>
<td>4.3 ± 2.1</td>
<td>4.7 ± 3.0</td>
</tr>
<tr>
<td>Total sample (n = 211)</td>
<td>5.2 ± 3.0</td>
<td>4.3 ± 2.1</td>
<td>5.4 ± 3.0</td>
<td>4.7 ± 2.7</td>
<td>5.1 ± 3.1</td>
<td>4.8 ± 2.3</td>
<td>4.6 ± 2.3</td>
<td>4.6 ± 2.3</td>
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</tbody>
</table>

*P < 0.05 (univariate spearmen correlation coefficients).
**P < 0.05 (t-tests) boys versus girls.

### TABLE 4—Independent Variables Included in the Forward Linear Stepwise Multiple Regression Model for the Fraction of Exhaled Nitric Oxide

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Non-standardized regression coefficient</th>
<th>Cumulative determination coefficient</th>
<th>P level</th>
<th>Standard error</th>
<th>1.64 residual standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls (n = 104)</td>
<td>No variable is included</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys (n = 107)</td>
<td>Constant 3.17682</td>
<td>0.0000</td>
<td>0.7715</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>1st second forced expiratory volume (l)</td>
<td>0.75009</td>
<td>0.0451</td>
<td>0.0281</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sample (n = 211)</td>
<td>Constant 3.91283</td>
<td>0.0000</td>
<td>0.6778</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>75% of forced vital capacity (l sec⁻¹)</td>
<td>0.46666</td>
<td>0.0236</td>
<td>0.0516</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
means of the measured and predicted FeNO mean values. Our results strongly suggest that existing FeNO norms need to be modified for North African/Arab children. Thus, we established a table of normal values according to age ranges. For North African children of any age, any FeNO value greater than 17.0 ppb may be considered abnormal. Finally, in an additional group of children prospectively assessed, we found no child with a FeNO higher than the threshold of 17.0 ppb or higher than the 95% CI ULN specific for each age range.

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Children Group

As for all the studies aiming to publish Fe\textsubscript{NO} norms,\textsuperscript{13–16} ours was not a random population sample. Some caution should be warranted when interpreting the results of cross-sectional studies in volunteers, because of a possible selection bias and cohort effects.\textsuperscript{34}

Thus, longitudinal studies analyzed by appropriate statistical models are necessary to correctly describe the functional changes associated with age.\textsuperscript{23} Although no statistical methods were used to choose the children, the number studied and the fact that many schools in different areas of Sousse were included give a
reasonable degree of confidence in the data. Our recruitment mode and subject age range were similar to previous studies having comparable aims than ours (Supplementary E. Table 1).

According to international recommendations, a large number of subjects (i.e., $n \geq 100$) is needed to ensure no significant difference between the published norms and the values from the local community. Our sample size ($n = 211$) appears to be satisfactory since the calculated one is 193 children.

Because of the frequency of asthma (7–10%) and atopy in children population, we have chosen healthy children for this study. The childhood asthma is a major cause of pediatric emergency department visit, representing 5–6%. Thus, the early detection of this disease by measuring an easy and reliable parameter (FeNO) is desirable.

Non-inclusion criteria, applied in the present study, were inspired of similar studies according to ATS/ERS guidelines. In contrast of Buchvald et al. study where outliers were defined as FeNO values above arithmetic mean $+ 2$ SD, we have fixed the cut-off for outliers to the arithmetic mean $+ 3$ SD (equivalent to 17 ppb for girls aged 11.0–13.9 years where the mean of FeNO is 5.7 ppb; Table 2). In addition, when applied in our sample (i.e., age range of 15–16 years, FeNO mean $\pm$ SD $= 5.6 \pm 4.0$), Buchvald et al. definition does not modify the number of our outliers.

The present study sample size ($n = 211$) appeared to be satisfactory when it is compared with those of other studies ($n = 657^{13}$; $n = 114^{14}$; $n = 661^{15}$; $n = 405^{16}$; Supplementary E. Table 1). We prospectively measured the FeNO in a second group of additional healthy children meeting the inclusion criteria of the present study. The validation group number ($n = 24$) seems small, but we think that it is sufficient to provide adequate validation since it is closer to those included in similar studies aiming to validate North African reference equations for spirometric, peak nasal inspiratory flow, and for 6-min walk distance data, respectively ($n = 28$), and $n = 41$. Our study is the first one that uses an evaluation group, to verify the reliability of children FeNO norms.

The exact definition of a “healthy” group is debatable in children but we avoided confounding clinical situations, according to the ATS recommendation. Our children were free from chronic disease, but 48 children (24 boys) showed overweight or obesity. However, as did other authors, children having thinness or obesity were not excluded. Cebella et al. showed that, in children, overweight or obesity was not associated with increased FeNO levels, but they were an independent risk factor for asthma and allergic sensitization. Other authors demonstrated that BMI in asthmatics may increase airway oxidative stress and could explain the BMI-related reductions in FeNO. Therefore, our group composition reflects this “healthy” population.

In similar studies (Supplementary E. Table 1), two questionnaires, the most recommended for epidemiological research, were used (ATS-DLD-78-C and ISSAC children’s questionnaires). In the present study, both were combined and used to evaluate children characteristics, with some modifications to fit the socio-cultural needs.

### Table 5—Fraction of Exhaled Nitric Oxide (FeNO) Norms: FeNO Data (ppb) in Different Age Groups ($n = 211$)

<table>
<thead>
<tr>
<th>Age range (yr)</th>
<th>Number of children</th>
<th>Mean $\pm$ standard deviation</th>
<th>95% confidence interval upper limit of normal</th>
<th>Minimum–maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0–5.9</td>
<td>21</td>
<td>4.2 $\pm$ 2.5</td>
<td>5.4</td>
<td>1.0–10.0</td>
</tr>
<tr>
<td>6.0–6.9</td>
<td>13</td>
<td>4.8 $\pm$ 2.0</td>
<td>6.0</td>
<td>1.0–8.0</td>
</tr>
<tr>
<td>7.0–7.9</td>
<td>20</td>
<td>4.7 $\pm$ 2.8</td>
<td>5.9</td>
<td>1.0–12.0</td>
</tr>
<tr>
<td>8.0–8.9</td>
<td>19</td>
<td>4.7 $\pm$ 2.2</td>
<td>5.8</td>
<td>2.0–11.0</td>
</tr>
<tr>
<td>9.0–9.9</td>
<td>16</td>
<td>3.9 $\pm$ 1.8</td>
<td>4.8</td>
<td>1.0–7.0</td>
</tr>
<tr>
<td>10.0–10.9</td>
<td>23</td>
<td>6.3 $\pm$ 2.9</td>
<td>7.5</td>
<td>2.0–14.0</td>
</tr>
<tr>
<td>11.0–11.9</td>
<td>20</td>
<td>5.5 $\pm$ 3.1</td>
<td>7.0</td>
<td>2.0–13.0</td>
</tr>
<tr>
<td>12.0–12.9</td>
<td>15</td>
<td>5.7 $\pm$ 3.6</td>
<td>7.7</td>
<td>2.0–17.0</td>
</tr>
<tr>
<td>13.0–13.9</td>
<td>9</td>
<td>4.2 $\pm$ 1.5</td>
<td>5.4</td>
<td>2.0–6.0</td>
</tr>
<tr>
<td>14.0–14.9</td>
<td>24</td>
<td>4.7 $\pm$ 2.5</td>
<td>5.7</td>
<td>2.0–12.0</td>
</tr>
<tr>
<td>15.0–16.0</td>
<td>31</td>
<td>5.6 $\pm$ 4.0</td>
<td>7.1</td>
<td>2.0–16.0</td>
</tr>
<tr>
<td>5.0–16.0 total sample</td>
<td>211</td>
<td>5.0 $\pm$ 2.9</td>
<td>5.4</td>
<td>1.0–17.0</td>
</tr>
</tbody>
</table>
Because environmental NO can reach high levels relative to those in exhaled breath, standardized techniques must prevent the contamination of biological samples with ambient NO. As recommended by the ATS/ERS, not withstanding which technique is used; ambient NO at the time of each test should be recorded. In the present study, mean ± SD (minimum–maximum) ambient NO concentration is 1.3 ± 1.3 ppb (0–4 ppb). Medisoft device has an absorption column with high capacities for detecting and eliminating ambient NO. Thus its function is not limited by the values of ambient NO.

As it is uncertain whether measurements need to be standardized for time of day (circadian rhythm effects), we were prudent and FeNO measurements were performed in the same period of the day (8 a.m. to 12 a.m.).

Interpretation of FeNO values relies upon comparison with predicted value available from published norms. To our knowledge, the present study is the first that reported FeNO norms for healthy North African children aged from 6 to 16 years. Therefore, there is a continuing need for such clinical research.

Non-Disease-Related Subject Factors Influencing FeNO Values

In the total sample, gender, anthropometric data, pubertal status, sports activity, and SEL did not significantly affect the FeNO. Only spirometric data influence significantly FeNO levels. These factors will be analyzed one by one in the following sections.

Gender effect: As in published studies (Supplementary E. Table 1), gender did not affect FeNO of Tunisian children. This factor could be reported after pubertal age. Additional information about the gender effect is detailed in the “Supplementary data.”

Age effect: As in two studies (Supplementary E. Table 1), age did not correlate with Tunisian children FeNO values (Table 4). Buchvald et al., in a population of 405 children, have found that the upper limit of the 95% CI was age dependent, ranging from 15.7 ppb at the age of 4 years to 25.2 ppb for adolescents. While some studies reported that FeNO increases with age (Supplementary E. Table 1), the mechanism for the age dependence of FeNO is largely unknown. Additional information about the age effect is detailed in the “Supplementary data.”

Body weight and BSA effects: In contrast of some children studies (Supplementary E. Table 1) or healthy adults studies, we have not reported a significant correlation between body weight or BSA and FeNO levels in either gender. As mentioned by Yao et al., a preliminary consensus reached between their study and that by Linn et al. is that the influence of weight on FeNO levels is relatively small.

Pubertal status effect: Pubertal status, a factor that had not been evaluated before, did not significantly affect the FeNO. It seems that hormonal modification did not affect the FeNO values.

Physical activity effect: Sports activity, a condition that had not been evaluated before, did not significantly affect the FeNO. During exercise, according to one report, FeNO increases, and this effect may last up to 1 hr. Others have reported that FeNO remains stable after exercise. It would seem prudent to avoid strenuous exercise for 1 hr before the measurement.

SEL effect: SEL, a factor that had not been evaluated before, did not significantly affect the FeNO. The effects of SEL on the spirometric variables are well documented in industrialized countries: a low SEL accelerates their decline and is associated with small airway obstruction.

Airway caliber effect: For the included boys and the total sample; respectively, only FEV1 (l) and only FEF25–75% (l sec−1) explain a slight but significant FeNO variability (Table 4). This result is not in agreement with previous published norms (Supplementary E. Table 1). However, it has been demonstrated that FeNO levels may vary with the airway caliber, perhaps because of a mechanical effect on NO output. Given that FEV1 correlated with FeNO only in boys (Table 4), one wonders whether boy’s larger airways produced more NO?

Why the Findings About the FeNO Determinants Are Not Consistent With Previous Literature?

There is much remaining variation that is unexplained, and several factors may be involved in addition to methodological factors, for example, subclinical airway inflammation of various causes, nutritional history, and race or genetic factors.

As FeNO values obtained with different devices are not directly comparable and may differ to a clinically relevant, as the device is used, we have adjusted our data according to Brooks et al. As can be seen (Supplementary E. Fig 3) and even after adjustment, our mean ± SD measured FeNO was significantly overestimated by the Canadian, the Taiwan, and the Finnish reference equations. Also, when compared with the adjusted values of the multicentre study, our mean
(95% CI ULN) measured FeNO remains significantly overestimated but only for the {10–13} and {14–16} age ranges. Therefore, care must be taken when comparing the present study FeNO results with those using different machines in different studies. Thus, the use of other studies FeNO norms may lead to misinterpretation of the FeNO values.

Among the published FeNO norms for children13–16 (Supplementary E. Table 1), none have proposed a method of interpreting the measured FeNO or have provided a prospective verification of their studied populations.

FeNO Norms and Interpretation

Due to the inadequacy of the published norms,13–16 we established local normal ranges adapted to our population. For practical and routine interpretation of FeNO, three ways were proposed.

A reference equation should include only easily measured anthropometric data that appears to influence FeNO. Spirometric parameters have been studied in our study and by some authors15,43 but were not retained for our and their final reference equations for a practical and simple interpretation. In the present study, no easily measured anthropometric data appear to explain FeNO variability. For that reason, we have not established a reference equation for FeNO and like in the study of Buchvald et al.16 (Supplementary E. Table 1), we have proposed local FeNO normal values (Table 5). In addition, compared to the published reference equations,13–15 we found significant differences between measured and predicted FeNO (Figs. 2 and 3). The measured FeNO of different age groups was compared with those measured in the multicentric study.16 In all instances, our mean (95% CI ULN) measured FeNO was significantly overestimated (Fig. 2). This can be explained by the racial factor13 and the method of measure (Medisoft vs. NIOX).33

Reliability of the Local FeNO Norms

The prospectively evaluated population demonstrated the problems of using normal ranges established in other populations. The reliability of the normal range we established was confirmed in the prospectively studied population, confirming the continuing need of establishing regional reference norms as stated by the ATS.12 This argues for the use of a specific reference norms in the present population. The implications of this for children with bronchial asthma may be considerable, resulting in a false-positive misdiagnosis of bronchial inflammation.

FeNO measurements offer a step forward in the assessment of airways disease. As an “inflammmometer,” FeNO provides the clinician with hitherto unavailable information regarding the nature of underlying airway inflammation, thus complementing conventional physiological testing, including the measurement of airway hyper-responsiveness. FeNO measurements are easy to perform, reproducible, and technically less demanding than induced sputum analysis. They are unreliable in current smokers and, when used diagnostically, in patients who have been taking inhaled or oral steroids recently.5

In conclusion, we have established reliable norms to interpret the results of FeNO in healthy North African children. The FeNO can easily be predicted according age-table ranges. Local FeNO norms enrich the global repository of FeNO norms the pediatrician can use to choose the most appropriate norms based on children’s location or ethnicity.

ACKNOWLEDGMENTS

Authors are thankful to directors of the Saladin primary school of Sousse and the Jawhara college of Sousse for their helping in the recruitment of children. Authors also wish to thank professors Lamia Boughammoura, Ahmed Abdelghani, and Iheb Bougmiza for their invaluable contribution in the preparation of the manuscript.

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