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Relationships of online exhaled, offline exhaled, and ambient nitric oxide in an epidemiologic survey of schoolchildren

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Field measurements of exhaled nitric oxide (FeNO) and ambient nitric oxide (NO) are useful to assess both respiratory health and short-term air pollution exposure. Online real-time measurement maximizes data quality and comparability with clinical studies, but offline delayed measurement may be more practical for large epidemiological studies. To facilitate cross-comparison in larger studies, we measured FeNO and concurrent ambient NO both online and offline in 362 children at 14 schools in 8 Southern California communities. Offline breath samples were collected in bags at 100 ml/s expiratory flow with deadspace discard; online FeNO was measured at 50 ml/s. Scrubbing of ambient NO from inhaled air appeared to be nearly 100% effective online, but 50–75% effective offline. Offline samples were stored at 2–8°C and analyzed 2–26 h later at a central laboratory. Offline and online FeNO showed a nearly (but not completely) linear relationship ($R^2 = 0.90$); unadjusted means (ranges) were 10 (4–94) and 15 (3–181) p.p.b., respectively. Ambient NO concentration range was 0–212 p.p.b. Offline FeNO was positively related to ambient NO ($r = 0.30$, $P < 0.0001$), unlike online FeNO ($r = 0.09$, $P = 0.08$), indicating that ambient NO artifactually influenced offline measurements. Offline FeNO differed between schools ($P < 0.001$); online FeNO did not ($P = 0.26$), suggesting artifacts related to offline bag storage and transport. Artifact effects were small in comparison with between-subject variance of FeNO. An empirical statistical model predicting individual online FeNO from offline FeNO, ambient NO, and lag time before offline analysis gave $R^2 = 0.94$. Analyses of school or age differences yielded similar results from measured or model-predicted online FeNO. Conclusions: Either online or offline measurement of exhaled NO and concurrent ambient NO can be useful in field epidemiology. Influence of ambient NO on exhaled NO should be examined carefully, particularly for offline measurements.
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Introduction

Exhaled nitric oxide (FeNO) concentration is an indirect measure of airway inflammation and oxidative/nitrosative stress, useful in diagnosis and management of asthma (American Thoracic Society, 1999, 2005; Kharitonov and Barnes, 2001, 2006; George et al., 2004; Ricciardolo et al., 2004; Zeidler et al., 2004; Taylor et al., 2006). Direct online measurement, with simultaneous recording of exhaled NO concentration and expiratory flow, is the "gold standard" technique. Offline measurement, with collection of exhaled breath in bags for later analysis, is more widely usable but allows less precision and quality control. Reasonable agreement has been demonstrated between offline and online measurements in laboratory-based studies, using a variety of techniques (Barreto et al., 2001; Jöbsis et al., 2001; Deykin et al., 2002; Kissoon et al., 2002; Linn et al., 2004). However, differences may persist even after careful matching of the online and offline methods for controlled exhalation and breath sampling (Tadaki et al., 2008). More often, faster expiratory flows are used offline than online, which may improve subjects' comfort, but complicates comparisons of data obtained by the different methods, as FeNO is strongly flow dependent.

We are conducting a longitudinal study of FeNO, intended to elucidate its relationship to long-term air pollution exposure and new-onset asthma, in a large population-based sample of children participating in the Asthma Incidence Risk Study (AIRS). The latter is part of the Children's Health Study, an ongoing prospective cohort study of environmental and genetic determinants of respiratory health in southern California children (Peters et al., 1999; Gauderman et al., 2004). FeNO has been measured annually in >2500 children at their schools, located in 13 communities with different levels and types of air pollution. Initially, measurements used an offline technique (Linn et al., 2004) with refrigerated storage/transport of samples and delayed analysis at a central laboratory. Portable equipment for

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standard online measurements became available and is now used to collect FeNO in field studies. To develop methods to include both online and offline FeNO in longitudinal analyses, we performed nearly concurrent online and offline measurements in about 15% of the AIRS population. This report presents statistical analyses comparing online and offline data, intended to predict online FeNO values that would have been measured in earlier years from the available offline data. The results may be applicable also to online–offline comparisons in other studies using similar techniques.

Methods

Informed assent and consent was obtained from each child and a parent respectively. The protocol was reviewed and approved by the University of Southern California Institutional Review Board. Questionnaire responses by parents were used to determine children's demographic characteristics. In the full-scale AIRS study, online FeNO tests were performed on >2500 subjects at 49 schools in 13 communities between October 2006 and June 2007. Tests were scheduled from mid-morning to early afternoon to avoid traffic-related peaks of ambient NO. Each community was visited at least twice in different seasons, to minimize confounding of location and season effects. Offline tests were added in a subsample of testing sessions selected to cover most of the geographic and seasonal range, including 1 or 2 testing days at each of 15 schools in 8 communities. A total of 386 children performed offline testing in the same session as their normally scheduled online testing, with on/off order divided about equally. The interval between online and offline testing was <1 h for 381 subjects and 1–4 h for 5 subjects.

FeNO measurement methodology is summarized here; an internet-accessible technical report (Linn et al., 2008) provides additional details. Online measurements were performed at 50 ml/s expiratory flow using EcoMedics CLD-88-SP analyzers, with DeNOx accessories to provide NO-free inhaled air (EcoPhysics Inc., Ann Arbor, MI, USA/Duernten, Switzerland), according to the manufacturer's instructions based on professional societies' recommendations (American Thoracic Society, 1999, 2005; Baraldi and DeJongste, 2002). The analyzers' sampling rate was ~330 ml/min. In each online test maneuver the subject took two or more preliminary tidal breaths and a nearly maximal inspiration from the DeNOx unit, then exhaled near 50 ml/s against the sampling head's fixed resistance, controlling flow by observing a color-coded analog display on a computer screen. Five maneuvers were recorded per subject. FeNO was represented by the mean of three American Thoracic Society (2005) acceptable plateau means with concentrations agreeing within 10%, or two within 5%. If the median FeNO of all ATS acceptable maneuvers was under 10 p.p.b., two maneuvers within 10% were accepted. Indoor ambient air was sampled approximately once per hour with the same analyzers, averaging the NO concentration over an interval of 10 s. An estimate of ambient NO concentration during each subject's test was made by linear interpolation between ambient measurements.

Exhaled breath samples for offline testing were obtained using Bag Collection and Sampling Kits and 1.5–l aluminized Mylar bags (Sievers Division, GE Analytical Instruments, Boulder, CO, USA). The manufacturer's instructions for breath collection at 100 ml/s expiratory flow after discarding deadspace air, based on American Thoracic Society (1999) recommendations, were followed. The subject took preliminary tidal breaths and a nearly maximal inspiration through the sampling kit's NO scrubber, then exhaled through the kit's fixed resistance, controlling flow by observing a mechanical pressure gauge marked to indicate the target rate. After observing the beginning of exhalation on the pressure gauge, the test technician counted off 3 s during which exhaled air was discarded (considered sufficient to clear deadspace, allowing some margin for timing or flow inaccuracy), then switched the exhaled air flow to the Mylar bag and collected approximately 1 l of breath. Each subject provided two bag samples; the one with lower NO concentration was taken as representative, as positive errors were far more likely than negative ones, according to previous experience here as well as prior literature reports and technical information from the sampling kit manufacturer (Linn et al, 2004, 2008). Bag samples of indoor ambient air, one unfiltered and one filtered through the sampling kit's NO scrubber, were collected repeatedly in each testing session, using a syringe to simulate a human subject's breathing; they were stored and analyzed like breath samples. Ambient NO at the time of each subject's offline testing was estimated by linear interpolation between ambient sample measurements. Between collection and analysis, bag samples were stored and transported on "blue ice" at 2–8°C. Lag times between collection and analysis ranged from 2 to 6 h for samples analyzed on the day of collection, and from 18 to 26 h for samples that had to be stored overnight before analysis. Bag samples were analyzed at ~24°C room temperature with a Sievers Model 280i NO analyzer, sampling at its standard rate of ~200 ml/min. Concentration *vs* time was recorded digitally using the analyzer's software. The concentration plateau for each sample was detected algorithmically and the median plateau concentration was taken as representative. A plot of each analyzed time/concentration profile was reviewed to confirm proper detection of the plateau. Sievers and EcoMedics analyzers were cross-compared with multiple certified span gases, plus breath and ambient air samples, on six occasions during the testing season. EcoMedics analyzers were calibration-checked weekly with zero and span gas; Sievers analyzers were checked before and after each analysis session.

Commercial software (SAS; SAS Institute, Cary, NC, USA; Excel, Microsoft Corp., Redmond, WA, USA) was used for data management and statistical modeling. Although demographic and clinical characteristics can influence FeNO, only actual NO measurements and potential artifacts were addressed in this modeling effort, as its goal was to relate one measurement method to another. Modeling was based only on the present data. (Although our earlier pilot study (Linn et al., 2004) appeared to provide reliable correction factors for offline FeNO based on lag time and initial concentration, applying the pilot-study-derived statistical model to present offline data gave biologically implausible results for some individuals. Thus, that model — based on laboratory testing plus very limited field testing — apparently could not account for the broader range of conditions encountered in a larger-scale field study.) Initial exploratory analyses indicated that date of testing, time of testing, and history of the breath sample bag were not significant influences, but that ambient NO and lag time were potentially significant influences on offline FeNO. They also showed that one school differed significantly ($P<0.001$) from all others with respect to the relationship between online and offline FeNO: online values were increased on average, but offline values were increased disproportionately more, suggesting that some or all samples had been affected by an unidentified positive interference. That school's data were excluded from statistical modeling, leaving 362 subjects from 14 schools. Models to predict each individual's online FeNO from offline FeNO were selected empirically by attempting to maximize the proportion of variance explained (R^2) while obtaining physiologically plausible predictions at the upper and lower extremes of the range. Simple linear or polynomial models gave implausible predictions at extremes; therefore spline-based piecewise linear modeling (Freund and Littell, 2000) and log transformations were adopted (Linn et al., 2008). Once an "optimum" model was found, it was further validated by performing analyses of variance to test differences between schools or between age groups, comparing results with actual measured online FeNO against results with model-predicted FeNO.

Results and discussion

Table 1 shows summary statistics for exhaled NO measurements and concurrent ambient NO estimates online and offline, and for lag time between offline breath collection and measurement. In the more polluted communities, ambient NO ranged widely and often exceeded typical FeNO. Both FeNO and ambient NO distributions were highly skewed, with a much wider range of values above than below the median, as shown in Figure 1. Variation in lag time reflected logistic issues: samples collected far from the laboratory could not be analyzed until the next day, whereas samples collected nearby might be analyzed either the same day or the next day. Thus, the distribution of lag times had modes near 4 and 24 h, and no values between 6 and 18 h. Differences in mean measured FeNO between schools were nonsignificant online, but highly significant offline, suggesting that artifacts influenced offline measurements differently at different locations.

Table 1. Unadjusted means and standard errors of NO measurements (p.p.b.) and lag times between offline collection and analysis (h) for entire sample and each school.

School	Online measurements					Offline measurements					
	N	FeNO mean[a]	SE	Ambient mean	SE	FeNO mean	SE	Ambient mean	SE	Lag mean	SE
302	28	11.9	3.3	2.2	5.2	7.2	1.7	3.0	4.0	20.2	0.9
614	25	17.6	3.5	0.1	5.5	9.5	1.8	0.8	4.2	3.6	0.9
616	22	12.8	3.7	0.3	5.9	7.0	1.9	1.4	4.5	17.4	1.0
703	24	11.6	3.6	0.5	5.7	6.4	1.8	1.8	4.3	21.8	1.0
707	26	22.3	3.4	2.8	5.4	13.2	1.7	4.9	4.1	24.0	0.9
814	24	15.1	3.6	62.1	5.7	9.6	1.8	36.9	4.3	4.9	1.0
815	11	10.6	5.3	10.1	8.4	8.7	2.7	11.3	6.3	16.5	1.4
905	25	14.6	3.5	17.9	5.5	8.4	1.8	15.2	4.2	3.3	0.9
906[b]	24	23.8	3.6	0.6	5.7	16.7	1.8	2.2	4.3	4.1	1.0
1202	26	13.2	3.4	38.0	5.4	10.0	1.7	38.2	4.1	13.9	0.9
1206	21	10.3	3.8	60.9	6.1	7.7	1.9	35.9	4.6	14.6	1.0
1301	26	18.5	3.4	12.5	5.4	10.3	1.7	11.1	4.1	21.5	0.9
1304	50	16.3	2.5	26.9	3.9	11.2	1.3	26.9	3.0	23.0	0.7
1402	23	14.9	3.6	121.5	5.8	12.9	1.8	78.8	4.4	23.9	1.0
1403	31	20.4	3.1	100.3	5.0	16.0	1.6	85.6	3.8	15.8	0.8
All	386	16.0	0.9	31.2	2.3	10.5	0.5	24.9	1.7	15.8	0.4

[a]Difference between schools in mean online FeNO is nonsignificant by analysis of variance ($P=0.17$). For all other variables in table, difference between schools is significant ($P<0.001$).
[b]School 906 data are included in above statistical results but are not used in subsequent predictions of online from offline FeNO. If school 906 is excluded, statistical significance/nonsignificance noted in (a) persists.

Table 2 shows pairwise correlations of NO and lag-time variables for 362 subjects. Online and offline FeNO showed a close relationship ($r = 0.95$). Online and offline ambient NO showed a similarly close relationship, even though both were estimated by linear interpolation, measurements were not simultaneous, and ambient concentrations might vary substantially over time. Lag time showed a weak but significant positive correlation with offline NO measurements, as expected because most breath or ambient samples gain NO slowly during refrigerated storage (Linn et al., 2004). (The exceptions are samples with initial NO concentrations near the upper end of the observed range, for which the NO–oxygen reaction rate is appreciable.) Offline FeNO showed a significant positive relationship to concurrent ambient NO (slope = 0.074, $r = 0.30$, $P < 0.0001$). Such a relationship might result from an acute biological effect of ambient pollution on FeNO, as has been reported previously in short-term longitudinal panel studies of asthmatic children (e.g. Mar et al., 2005; Delfino et al., 2006). Here, however, the relationship must have been at least partly artifactual, because concurrent online measurements of FeNO and ambient NO showed less relationship (slope = 0.033, $r = 0.09$, $P = 0.08$). Figure 2 illustrates the relationships of offline and online FeNO to the corresponding ambient measurements. The artifact may be explainable in that offline breath kit NO scrubbers did not completely remove ambient NO from inhaled air. In laboratory tests with slow steady flow (either 200 ml/min using the NO analyzer pump only, or ~10 l/min using a small diaphragm pump), the scrubbers removed as much as 97% of ambient NO. However, in collections at schools using a syringe to simulate the breathing of a human subject with 3 l vital capacity, with unsteady "inspiratory" flow averaging roughly an order of magnitude faster than in laboratory tests, scrubbers typically removed only 50–70% of ambient NO, and filtered-sample concentrations correlated with unfiltered (ambient) concentrations (see Figure 3). Assuming that scrubbers behaved similarly with actual human subjects, and given that inhaled NO uptake in proximal airways is slow relative to inspiratory time (DuBois *et al.*, 1999), some ambient NO that passed through the scrubber would enter the breath sample, and an artifactual positive relationship of ambient NO to offline FeNO would result. This effect would be magnified if deadspace was not completely cleared before starting breath collection — a possibility that cannot be ruled out in all cases. By contrast, ambient NO was effectively removed from air inhaled in online testing: periodic spot checks of DeNOx output consistently showed < 1 p.p.b. NO, regard-

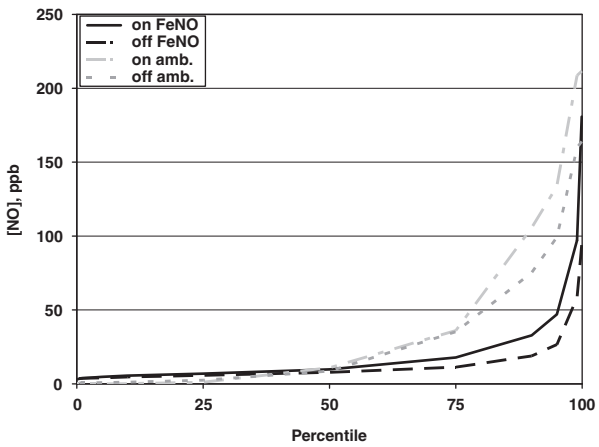

Figure 1. Cumulative frequency distributions of measured FeNO concentrations, and concurrent ambient NO concentrations estimated by interpolation from periodic ambient measurements at the testing location.

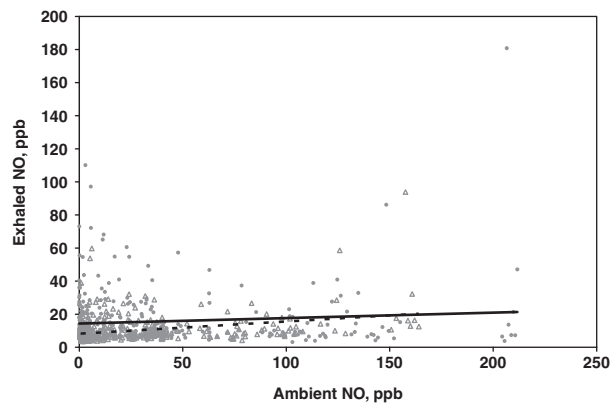

Figure 2. Exhaled NO *vs* ambient NO: individual data and best-fit linear relationships for 362 subjects, excluding school 906. Open triangles and broken line represent offline data. Solid dots and solid line represent online data. Positive linear relationship is significant offline ($P < 0.0001$) but not online ($P = 0.08$).

Table 2. Pairwise correlations and (P-values) of exhaled NO, ambient NO, and lag time for 362 subjects, excluding school 906.[a]

	Amb. NO online	FeNO offline	Amb. NO offline	Lag
FeNO online	0.093 (0.08)	0.950 (<.0001)	0.092 (0.08)	0.069 (0.19)
Amb. NO online		0.283 (<.0001)	0.957 (<.0001)	0.089 (0.09)
FeNO offline			0.300 (<.0001)	0.149 (0.005)
Amb. NO offline				0.152 (0.004)

[a]If 24 subjects from school 906 were included, correlation of ambient NO online with lag became significant ($r = 0.140$, $P = 0.006$) and correlation of offline FeNO with lag lost significance ($r = 0.065$, $P = 0.20$); otherwise, correlations were similar to those in table.

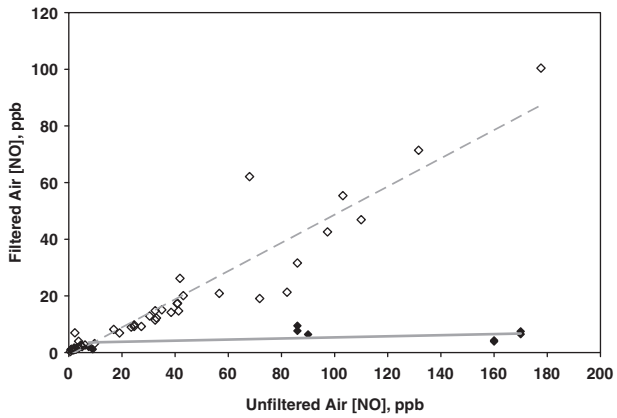

Figure 3. NO concentration in air filtered by breath sampling kit, as a function of ambient NO concentration. Solid symbols and solid regression line: laboratory tests with filtered air collected at steady flow $<=10$ l/min; regression slope $=0.02$. Open symbols and dashed regression line: sample collections during field testing, with usual inhalation pattern simulated by 3-liter syringe (typical flow ~ 100 l/min); regression slope $=0.5$.

less of ambient concentration. Furthermore, electronic determination of the NO plateau online eliminated the possibility of deadspace interference.

A spline-based piecewise linear model yielded plausible predictions of online FeNO throughout the range of offline FeNO, and increased R^2 to 0.91, compared to 0.90 for the linear relationship of measured online to offline FeNO. However, residuals from the spline-based model showed a negative relationship to ambient NO ($P<0.0001$) and to lag time ($P=0.003$), reinforcing the impression of artifactual influences on offline measurements. We therefore developed a model with adjustments for those variables. After unsuccessful efforts with simpler models, we computed a proportionality factor — a ratio of measured online to offline FeNO — for each subject, and formulated a regression model to describe how the proportionality factor varied with offline FeNO, ambient NO, and lag time. The model-predicted proportionality factor would be multiplied by offline-measured FeNO to predict online FeNO. Expressing ambient NO as a proportion of exhaled NO, and allowing different slopes for ambient concentrations above and below exhaled concentrations, gave the best fit to proportionality factor data. This suggested a small percentage of ambient air admixture in offline breath samples: ambient NO concentrations exceeding exhaled NO concentrations would cause proportionate increases in measured FeNO compared to true exhaled concentrations; whereas with ambient concentrations lower than exhaled, smaller proportionate decreases in measured FeNO would result, because of restricted range on the low side. In dealing with the lag-time effect, we obtained the best fit by making separate predictions for breath samples analyzed on the day of collection ("same day") and those analyzed on the day after collection ("overnight"). Within either group, the effect of lag time was nonsignificant and could be excluded from the model. To help normalize distributions, variables were natural log-transformed for regression analysis; then predicted values were exponentiated to yield predicted proportionality factors.

The final prediction equations, in the form ON = OFF × (proportionality factor), were as follows:

$$\mathrm{ON} = \mathrm{OFF} \times (\exp(0.18221 + 0.14062 \times \log(\mathrm{OFF}) - 0.13868 \times \log(\max(1, \mathrm{AMB}/\mathrm{OFF})) - 0.03596 \times \log(\min(1, \mathrm{AMB}/\mathrm{OFF})))) \text{ for same-day samples;}$$

$$\mathrm{ON} = \mathrm{OFF} \times (\exp(0.10302 + 0.15793 \times \log(\mathrm{OFF}) - 0.27801 \times \log(\max(1, \mathrm{AMB}/\mathrm{OFF})) - 0.04386 \times \log(\min(1, \mathrm{AMB}/\mathrm{OFF})))) \text{ for overnight samples.}$$

In these equations, ON represents predicted online FeNO, OFF represents measured offline FeNO, max() indicates the larger quantity in parentheses, min() indicates the smaller quantity in parentheses, and AMB represents estimated ambient NO at the time of offline collection. The positive slopes for log(OFF) were significant ($P<0.005$), indicating that the proportionality factor tended to increase with increasing offline FeNO. The latter observation is consistent with expectations, assuming that elevated FeNO commonly reflects increased NO excretion from proximal airways (as occurs, for example, in bronchial inflammation due to asthma), which increases the flow-dependent component of exhaled NO. This should have a larger effect on online than offline measurements because of slower expiratory flow online. Negative slopes were highly significant ($P<0.0001$) for the "max" terms (AMB $>$OFF), as appropriate to compensate for the presumed artifactual rise in offline (but not online) FeNO at high ambient concentrations due to ambient air admixture. The more negative slope for overnight samples may reflect the second important source of positive artifacts in typical offline samples — slow NO concentration gain during storage — which would become more influential with longer storage. The smaller negative slopes for the "min" terms (AMB $<$OFF) did not reach statistical significance, but were close enough to retain in the model ($P\sim 0.15$). Figure 4 plots observed online FeNO against predictions from the second model. The overall R^2 was 0.94. Distributions of observed and predicted online FeNO were reasonably similar over the entire range (see Table 3).

As a further check of this model's validity, mean differences between schools were tested for significance, first for measured online FeNO, then for predicted online FeNO. Figure 5 shows the results. For most schools, means and standard errors of predicted FeNO closely matched those for measured FeNO; the correlation between observed and predicted means was 0.94. As mentioned previously, differences between schools in measured online FeNO were not significant ($P=0.26$ for 362 subjects), whereas differences in measured offline FeNO were highly significant ($P<0.001$). Assuming that online measurements more

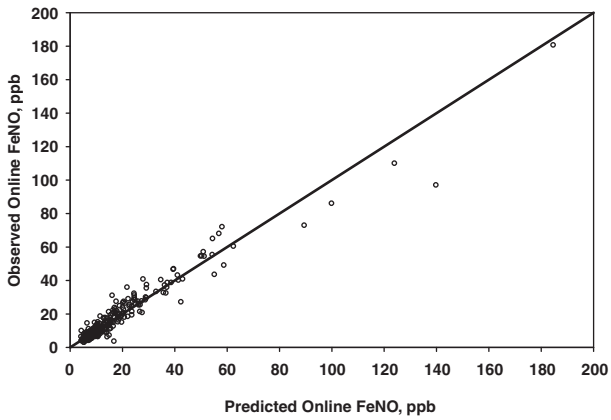

Figure 4. Measured online FeNO *vs* value predicted by second model (adjusted for ambient NO and lag-time artifacts). Solid line, line of identity.

Table 3. Percentile distributions of observed online FeNO and online FeNO predicted by second model, for 362 subjects excluding school 906.

Percentile	Observed	Predicted
100	180.8	184.5
99	86.2	99.9
95	43.3	41.3
90	30.1	27.5
75	17.5	16.0
50	9.8	9.9
25	7.0	7.3
10	5.7	5.9
5	4.9	5.4
1	3.9	4.4
0	3.2	4.1

accurately reflect subjects' physiology, the offline significance is probably invalid (i.e. artifactual not physiological). With predicted online data, school differences were marginally nonsignificant ($P = 0.068$). This result argues that prediction of online FeNO by the second model substantially reduces the likelihood of "false-positive" conclusions concerning group differences, which may occur with unadjusted offline data.

In still another validity check, subjects were grouped by age range and group differences were tested for significance. Table 4 shows results. With online measured data, group differences were modestly significant, primarily because of lower values in the youngest group. With predicted online data, estimated means were reasonably similar to those for measured data, and their differences fell just short of significance. With measured offline data, the proportionate differences were slightly smaller and less close to significance. This argues that online FeNO data predicted by the second model are more likely to detect true group differences as statistically significant, in comparison with unadjusted offline FeNO data.

Conclusions

Either online or offline measurement of FeNO, with concurrent measurement of ambient NO using the same instruments, may be useful in field studies of respiratory health and short-term air pollution exposure. Despite improved portability of current-generation online NO analyzers, economic and logistic constraints may still dictate offline measurement in some circumstances. Regardless of the FeNO measurement technique, it is important to measure the influence of short-term variation in NO-containing ambient pollution on the results, to detect either real (biological) acute changes in response to pollution exposure, or artifactual changes due to ambient NO admixture. With offline measurement, artifacts related to the sample container, sample storage conditions, and duration of storage also need to be addressed. In general, effects of these interferences appear to be small in comparison with the large interindividual variation in FeNO. After adjustment for artifacts, offline measurements at 100 ml/s expiratory flow with deadspace discard can yield reasonably accurate predictions of online FeNO as measured at 50 ml/s expiratory flow. The latter technique is now the *de facto* standard method for clinical testing; thus, reporting results in terms of this method will allow more straightforward (albeit still imprecise) comparisons across different studies. The prediction model reported here may be applicable in other studies using similar offline measurement techniques. However, in light of possible inconsistencies even among different online measurements, and the wide range of possible artifacts, any new study involving offline measurements ideally should include a comparison with "standard" online methods, specific to its own conditions.

In summary, online testing at 50 ml/s expiratory flow, with the subject breathing NO-free air, is the preferred method for measuring FeNO in epidemiologic surveys as well as in routine clinical testing. Offline testing with commercially available equipment is inherently less precise, because of inability to document accurate flow, inability to capture the breath sample at the optimum plateau concentration consistently, possible contamination of the sample by ambient NO, changes in sample concentration between collection and analysis, and possibly other artifacts related to the sample bag. Despite these problems, this study shows that offline testing can be highly effective in large-scale field surveys and in other situations not amenable to online testing.

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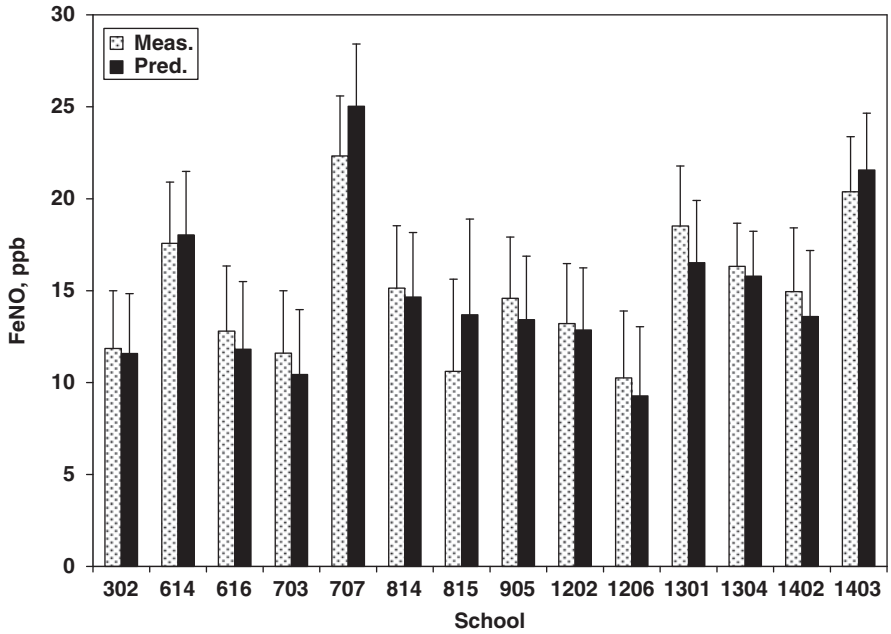

Figure 5. Differences between schools in measured and predicted online FeNO as estimated by analysis of variance. Bar indicates mean; flag indicates standard error.

Table 4. Results from analyses of variance.

	Mean (Standard error)			*P*-value
	Age <10 (32%)	Age 10–11 (55%)	Age ⩾11 (13%)	
Measured Online	12.2 (1.5)	17.2 (1.2)	16.0 (2.4)	0.036
Predicted Online	12.3 (1.6)	16.5 (1.2)	17.0 (2.5)	0.092
Measured Offline	8.8 (0.8)	10.9 (0.6)	10.3 (1.2)	0.123

Comparisons between age groups of measured FeNO and FeNO (in p.p.b.) predicted by second model.

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