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SHORT COMMUNICATION

Sputum induction with hypertonic saline reduces fractional exhaled nitric oxide in chronic smokers and non-smokers

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KEYWORDS

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Summary

Background: Nitric oxide (NO) measurements in exhaled air and hypertonic saline-induced sputum are commonly used biomarker sampling methods of the lower airways. Both sampling methods have been validated in asthmatic patients and healthy controls, however, data from chronic smokers are scarce.

Objectives: To evaluate the reproducibility and differences in fractional exhaled NO (FeNO) values in asymptomatic chronic smokers and healthy, non-smoking controls. Furthermore, to test the effect of hypertonic saline sputum induction (SI) on FeNO levels in both study groups.

Methods: 16 asymptomatic chronic smokers and 16 non-smokers participated in this study. Baseline FeNO and forced expiratory volume in 1 s (FEV₁) were recorded pre- and 30 min post NaCl 4.5% SI (3 × 5 min) on 2 study days (±2 h; 4–10 days apart). Mixed ANOVA was used to estimate the intra-subject Coefficient of Variation (CV) % over days; changes in FeNO and FEV₁ values before and after SI, were analyzed by a Student's paired *t*-test. The difference between smokers and non-smokers was estimated by a Student's *t*-test.

Results: On day 1, FeNO values in smokers were significantly lower than in non-smokers, 10.6 ppb, and 18.4 ppb, respectively, (42% difference, *p* = 0.0028, 95% CI: –59%, –19%). In both study groups, FeNO measurements were reproducible, with an intra-subject CV of 27.2% and 19.2%, for smokers and non-smokers, respectively. SI significantly decreased FeNO levels in both study groups on day 1. In smokers, there was a mean reduction in FeNO of almost 37% (*p* = 0.0045,

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95% CI: -53% , -14%), and in non-smokers a mean decrease of almost 37% (95% CI: -53% , -14% ; $p = 0.0045$). In both study groups SI did not affect FEV₁ ($p > 0.94$).

Conclusions: Our data extend previous findings in asthmatics and healthy controls to asymptomatic chronic smokers: 1. FeNO measurements are reproducible in both smokers and non-smokers; 2. baseline FeNO levels in chronic smokers are lower than in non-smokers and 3. sputum induction by hypertonic saline reduces FeNO levels in both study groups, without affecting lung function.
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Introduction

Sputum induction by hypertonic saline (SI) and fractional exhaled nitric oxide (FeNO) are validated, commonly used non-invasive biomarker sampling methods of the lower airways.^{1,2} FeNO measurements are increasingly applied for diagnosis and monitoring of asthma.³ Furthermore, both methods are often used as complementary research tools to assess the airway inflammation in response to interventions with (novel) anti-inflammatory therapeutic modalities.⁴ However, there is evidence that sampling methods sometimes interfere and thus may affect the levels of biomarkers.⁵ So far, two published studies have addressed the effect of SI on FeNO values in asymptomatic atopic subjects and asthmatic patients and showed a maximal decrease in FeNO directly post-induction with still a substantial decrease up to 4 h after SI.^{6,7} In this study population, FeNO levels were reproducible and unrelated to the initial SI-induced decrease in FEV₁.^{6,7} So far, few data have been published on chronic smokers.^{8,9} Therefore, we tested the reproducibility and differences in FeNO levels between asymptomatic chronic smokers and healthy non-smokers. Furthermore, we investigated the effect of SI on FeNO levels in both study groups.

Methods

Subjects

The study population consisted of two groups: 16 asymptomatic chronic smokers with a smoking history of at least 10 pack-years (8F/8M; 32–52 years) and 16 healthy non-smokers (8F/8M; 30–49 years) who had not smoked for at least 12 months prior to study enrolment and who had a total smoking history of less than 5 pack-years. For the smoker group, the last cigarette was smoked at least 1 h before any study procedure. All subjects had no history of relevant lung disease or any respiratory tract infection for at least 4 weeks before the start of the study. All subjects gave written informed consent. The study was approved by the Ethics Committee of Leiden University Medical Centre, Leiden, Netherlands.

Study design

The study comprised two study days, 4–10 days apart. On each study day, FeNO was measured approximately 55 min before and 30 min after the SI-procedure. All assessments were performed at the same time of the day (± 2 h). This study was conducted as part of a larger biomarker study; the focus of this manuscript is on methodological issues related to the interaction of SI on FeNO levels.

Fractional exhaled nitric oxide (FeNO)

FeNO measurements were performed by a chemiluminescence analyser (Ecomedics CLD88sp, Ecomedics, Duernten, Switzerland) according to current guidelines.¹ Briefly, after a deep inhalation of NO-free air, subjects exhaled for approximately 10 s against a resistance at a stable flow of approximately 50 mL/s. The mean of the first three technically acceptable measurements (within 10%) were included in the analysis and expressed in parts per billion (ppb).

Pulmonary function tests

Spirometry was performed according to standardized protocols by a calibrated spirometer (V_{\max} Spectra Sensor Medics; Cardinal Health, Houten, The Netherlands)¹⁰ connected to a personal computer. The mean of the two out of three (within 5%) highest, technically satisfactory forced expiratory volume in 1 s (FEV₁) measurements was included in the analysis.

Hypertonic saline sputum induction

Sputum was induced by hypertonic saline (4.5% NaCl) nebulised by an ultrasonic nebulizer (DeVilbiss Ultra NEB 2000, Somerset, PA, USA) according to current guidelines during three periods of 5 min each.² Spirometry was performed 7 min after each SI-period.

Analysis

The reproducibility of the FeNO levels in both study groups was assessed on log-transformed data by a Mixed Analysis of Variance (ANOVA) to estimate the intra-subject Coefficient of Variation (CV). The differences in FeNO levels between both study populations were analyzed with a Student's *t*-test and the effect of inhaled NaCl 4.5% on FeNO and FEV₁.

Table 1 Subjects' baseline characteristics.

	Non-smokers	Smokers
Number of subjects	16	16
Age (years)	41 (30–49)	41 (32–52)
Gender (M/F)	8M/8F	8M/8F
Height (meters)	1.75 (1.56–1.93)	1.73 (1.58–1.85)
Weight (kg)	77.5 (51.8–110.2)	73.1 (46.5–100.5)
BMI (kg/m ²)	25.1 (21.1–29.5)	24.3 (18.6–30.0)
FEV ₁ (L)	3.66 (2.75–5.16)	3.55 (2.38–4.64)
FEV ₁ (% predicted)	102.9 (78–119.6)	102.7 (82.1–121.1)
FVC (L)	4.78 (3.21–6.42)	4.46 (3.15–6.38)
FVC (% predicted)	105.1 (82–131)	111.7 (76–127)

Values presented as mean (range).

Table 2 Effect of SI (NaCl 4.5%) on FeNO and FEV₁ values.

Study population/parameters	Day 1			
	Pre NaCl 4.5%	30 min post NaCl 4.5%	Difference (95% CI)	p-value
<i>non-smokers</i>				
FeNO (ppb)	18.4	12.0	-34.9% (-57.3%, -0.6%)	<i>p</i> = 0.047
FEV ₁ (L)	3.66	3.68	0.02 (-0.38, 0.41)	<i>p</i> = 0.94
<i>smokers</i>				
eNO (ppb)	10.6	6.7	-36.6% (-53.2%, -14.2%)	<i>p</i> = 0.0045
FEV ₁ (L)	3.56	3.55	-0.01 (-0.52, 0.49)	<i>p</i> = 0.95
Study population/parameters	Day 2			
	Pre NaCl 4.5%	30 min post NaCl 4.5%	Difference (95% CI)	p-value
<i>non-smokers</i>				
FeNO (ppb)	15.9	12.3	-22.6% (-50.5%, 21.1%)	<i>P</i> = 0.25
FEV ₁ (L)	3.56	3.68	0.12 (-0.31, 0.57)	<i>P</i> = 0.56
<i>smokers</i>				
FeNO (ppb)	11.1	8.5	-23.9% (-43.5%, 2.6%)	<i>P</i> = 0.072
FEV ₁ (L)	3.49	3.53	0.04 (-0.44, 0.52)	<i>P</i> = 0.86
Coefficient of variation (CV%) for FeNO (pre NaCl 4.5%)		non-smokers		19.2%
		smokers		27.2%

CI = confidence interval. FeNO values in geometric means.

within both study groups was analyzed with the paired Student's *t*-test. Results were back-transformed to ratios and expressed as percentage difference.

Results

Study subjects

The study groups were well-matched with no statistically significant differences in baseline characteristics between the two groups (Table 1).

Reproducibility and difference in FeNO between study groups

The intra-subject mean CV for baseline FeNO measurements was 19.2% and 27.2% for non-smokers and smokers, respectively (Table 2). Mean FeNO was significantly lower in smokers compared with non-smokers (Table 2).

Effect of sputum induction on FeNO and FEV₁

On day 1, SI decreased FeNO levels in non-smokers by on mean 35% (95% CI: -57%, -0.6%; *p* = 0.047) and in smokers by on mean 37% (95% CI: -53%, -14%; *p* = 0.0045) (Table 2). SI did not affect FEV₁ in either study group.

Discussion

In line with previous observations in allergic asthmatics, we found reproducible FeNO levels in asymptomatic chronic smokers and healthy non-smoking controls. In smokers, FeNO levels were generally lower than in non-smokers and within similar ranges as previously reported.¹¹ Similarly to previous

observations in allergic asthmatics,⁶ hypertonic saline decreased FeNO levels in both study groups without affecting FEV₁. Therefore, our findings confirm and extend previous data.^{5-7,12}

The sputum inductions in our study were performed according to standardized procedures¹ in age- and gender-matched populations, while in the smokers the time between smoking and any measurements was kept within the same ranges.¹² Hence, the lack of statistical significance between both study groups and pre- and post-SI on study day 2 is most probably due to a larger variability of the FeNO values in a small sample size, possibly caused by external factors.

In line with previous studies we found lower FeNO levels in smokers compared with non-smokers.¹² It appears that smoking inhibits NO-formation from inducible nitric oxide synthase in epithelial lung cells.¹³ Furthermore, NO-synthesis may be reduced by negative feedback as a result of high NO-concentrations in cigarette smoke,¹² NO-oxidation or interaction with other molecules present in tobacco smoke.¹⁴

In conclusion, FeNO levels in chronic smokers were found to be reproducible and generally lower than in healthy non-smokers. Sputum induction reduced FeNO in both study populations without affecting FEV₁. Our data extend previous observations in allergic asthmatics to chronic smokers. In view of the interference of sputum induction with FeNO measurements: FeNO should be measured before sputum induction.

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Conflicts of interest

None of the authors has a conflict of interest with regard to the study conduct or the data presentation of this study.

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