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Smoking effect on exhaled nitric oxide (NO) flow-independent NO exchange parameters

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ABSTRACT

Background: It is a well-known fact that smoking is associated with a reduction in exhaled nitric oxide (NO) levels. There is, however, limited knowledge relating to the smoking-induced changes in the production or exchange of NO in different compartments of the airways.

Material and methods: The study comprised 221 adult subjects from the European Community Respiratory Health Survey II who were investigated in terms of their exhaled NO, lung function, IgE sensitisation and smoking habits. The following parameters were determined using the extended NO analysis: Caw_{NO} (mean airway tissue concentration of NO), $Calv_{NO}$ (mean alveolar concentration of NO), Daw_{NO} (airway transfer factor for NO) and $FE_{NO\ 0.05}$ (fractional exhaled concentration of NO at a flow rate of 50 mL s^{-1}). $J'aw_{NO}$ (total maximum flux of NO in the airways) was calculated by $Daw_{NO}x(Caw_{NO}-Calv_{NO})$.

Results: Current smokers (n=35) had lower values (geometric mean) of $FE_{NO0.05}$ (14.0 vs 22.8 ppb, $p<0.001$), Caw_{NO} (79.0 vs 126 ppb, $p<0.001$) and $J'aw_{NO}$ (688 vs 1153 pL s^{-1}, $p=0.001$) than never-smokers (n=111). Ex-smokers (n=75) were characterised by lower $FE_{NO0.05}$ (17.7 vs 22.8 ppb, $p=0.02$) and $J'aw_{NO}$ values (858 vs 1153 pL s^{-1}, $p=0.02$) than never-smokers. These relationships were maintained after adjusting for potential confounders (gender, age, height, IgE sensitisation and FEV_1) and, in this analysis, a negative association was found between current smoking and $Calv_{NO}$ ($p=0.004$). Snus consumption (n=21) in ex-smokers was associated with an increase in Daw_{NO} ($p=0.04$) and a reduction in Caw_{NO} ($p=0.004$), after adjusting for potential confounders. Passive smoking was associated with a higher $Calv_{NO}$

(p=0.008).

Conclusions: Using extended NO analysis, it was possible to attribute the reduction in exhaled NO levels seen in ex- and current smokers to a lower total airway NO flux in ex-smokers and reduced airway and alveolar NO concentrations in current smokers. The association between snus (oral tobacco) use and reduced NO concentrations in the airways and increased NO transfer from the airways warrants further studies.

Abbreviations: NO, nitric oxide; $FE_{NO\ 0.05}$, fractional exhaled concentration of NO at a flow rate of 50 mL s^{-1}; Caw_{NO}, airway tissue concentration of NO; Daw_{NO}, NO airway transfer factor; $Calv_{NO}$, alveolar concentration of NO; $J'aw_{NO}$, total maximum flux of NO in the airways; NOS, nitric oxide synthase; FEV_1, forced expiratory volume in 1 second; NNK, N-nitrosamine 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone; BH_4, tetrahydrobiopterin

INTRODUCTION

A reduction in exhaled nitric oxide (NO) levels was first observed in smokers more than ten years ago (1;2) and the effect is found after both acute and chronic exposure to smoking (3). Passive smoking has also been found to reduce the levels of exhaled NO in healthy subjects (4) and asthmatic children (5). Smoking cessation is accompanied by an increase in exhaled NO levels (6) and, in one report, NO levels normalised after smoking cessation (7).

The possible mechanisms by which exhaled NO levels are reduced in smoking subjects are a potential negative feedback mechanism of the NO from the cigarette smoke that could lead to the down-regulation of nitric oxide synthase (NOS) in the lungs (8;9), an inadequate supply of co-factors necessary for NO production, such as tetrahydrobiopterin (BH_4) (10), or an increase in the breakdown of NO (11;11;12).

By modelling NO exchange dynamics, it is possible to obtain a greater insight into the two NO-producing compartments, the airways and alveoli, which are characterised by two or three flow-independent NO exchange parameters, depending on the model that is used. A review article on this topic has been published recently (13). The few studies investigating the effects of smoking on flow-independent NO exchange parameters indicate that smoking is related to a lower total maximum NO airway flux ($J'aw_{NO}$) (7) (14) and to a lower mean airway tissue concentration of NO (Caw_{NO}) (15). There is, however, very limited information on the dose-response relationship and the effect of past smoking and passive smoking on exhaled NO levels and flow-independent NO exchange parameters.

The aim of the present investigation was to study the effect of past, current and passive smoking on exhaled NO in a general population sample using flow-independent NO exchange parameters.

MATERIAL AND METHODS

Population

The subjects in this study were participants in a follow-up of the European Community Respiratory Health Survey (ECRHS), which was performed in Uppsala in 1990-1991 (16). Of the 622 subjects in the random sample of the ECRHS, 517 were re-investigated nine years later (1999-2000) in the European Community Respiratory Health Survey II (ECRHS II) (17). The majority of the subjects who were re-investigated (n=368) were seen at the hospital for a clinical examination, while the remaining 149 subjects only participated in a telephone survey, usually because they had moved outside the study area between the two surveys.

Of the 368 subjects who attended the clinical examination, 225 (61%) were also willing to perform exhaled NO measurements. In four of the subjects, we lacked information about their current smoking status and the present investigation therefore included 221 subjects.

Questionnaires

The ECHRS II main questionnaire (www.ecrhs.org) (17) was used to obtain information about symptoms, diagnoses, smoking history and habits. Additionally, we used information about snus (oral tobacco) consumption from the sleep questionnaire included in the Respiratory Health in Northern Europe study (RHINE) (www.rhine.nu) (18).

Measurements of exhaled NO

The NO measurements were performed according to American Thoracic Society (ATS) recommendations, apart from the use of three additional flows (5, 100 and 500 mL s^{-1}) and no vital capacity manoeuvre, as a deep breath with slow inhalation was found to be sufficient (19).

The system used for NO measurements was a computer-based, single-breath NO system from Nitrograf AB, Hässelby, Sweden, which used a chemiluminescence analyser (Sievers NOA 280, Sievers, Boulder, CO, USA). The system was calibrated using a mixture of 460 ppb NO in nitrogen (AGA AB, Lidingö, Sweden) and the zero was set by feeding synthetic air (AGA AB) into a 2L canister filled with Purafil II chemisorbant with purakol (Lindair AB, Ljusne, Sweden). The flow sensor was calibrated in the range of 0-0.6 L sec^{-1} (Dry Cal DC-2 flow calibrator, BIOS International, Pompton Plains, NJ, USA). Checks of the calibration and flow rate of the sampling system were made on a daily basis and the zero was controlled before each measurement. The expiratory pressure for all subjects was between 5 and 20 cm H_2O in order to exclude a NO contribution from the nasal cavity. A mean value of three breaths (or two if the NO concentrations were identical from the two breaths) was used for statistical analysis.

Application of the extended NO analysis

The extended NO analysis has been previously described and validated (15). Using the values of fractional exhaled nitric oxide (FE_{NO}) collected at three different flow rates (5, 100 and 500 mL s^{-1}) and an iteration algorithm, it calculates the three flow-independent NO exchange parameters confined to the two compartments: conducting airways, which are characterised by the mean airway tissue concentration of NO (Caw_{NO}) and NO airway transfer factor (Daw_{NO}), and alveoli, characterised by a mean alveolar concentration of NO ($Calv_{NO}$). A

fourth variable, $J'aw_{NO}$, was also used. $J'aw_{NO}$ represents the total maximum flux of NO in the airway compartment and is calculated by $Daw_{NO}x(Caw_{NO}-Calv_{NO})$. The reason for including $J'aw_{NO}$ in our study was that it provides a global airway compartment description. The fractional exhaled nitric oxide value at a flow rate of 50 mL s^{-1} ($FE_{NO\ 0.05}$) was used as a measure of the overall exhaled NO concentration. We chose to use the $FE_{NO\ 0.05}$ value in order to have a reference value for the other studies and to comply with ATS recommendations (20).

Lung function

Forced expiratory volume in one second (FEV_1) was measured using a dry rolling seal spirometer system (Sensor Medics 2130, Sensor Medics, Anaheim, California, USA). Up to five technically acceptable blows were determined. The ATS recommendations were followed (21). The predicted values for forced expiratory volume in one second (FEV_1) were calculated on the basis of the European Coal and Steel Union reference values (22).

IgE sensitisation

Blood samples were collected for the measurement of total and specific serum IgE using the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). Specific IgE was measured against *Dermatophagoides pteronyssinus*, cat, timothy grass and *Cladosporium herbarum*. The detection of specific IgE of > 0.35 kU/l was used as a definition of sensitisation to a specific allergen. ***IgE sensitisation*** was defined as sensitisation to at least one of the investigated allergens.

Tobacco use

Information on smoking history was collected by administering a questionnaire on each occasion. For those who answered "yes" to the lead question ("Have you ever smoked for as long as a year?"), additional questions were asked about age at starting, amount smoked currently, whether they had stopped or cut down and amount smoked previously. Based on this information, the subjects were classified as ***never-smokers***, ***ex-smokers*** and ***smokers***. The average number of cigarettes smoked per day was used to quantify exposure in current smokers. Lifetime exposure to smoking was calculated as pack years. ***Latency*** was defined as being the period of time (in years) since ex-smokers had stopped smoking.

Never- and ex-smokers who answered in the affirmative to the question: "Have you regularly (most days or nights) been exposed to tobacco smoke in the last 12 months?" were classified as being ***passive smokers***.

Snus consumption was registered as a yes/no answer, without recording information about the amount of consumption.

Statistical methods

Statistical analyses were performed using STATA 8.0 software (Stata Corp., 2001, Texas, USA). NO values, average daily cigarette consumption and pack-years consumption were log-transformed before analysis. Unpaired t-test was used for comparisons between ex-smokers with and without snus consumption and in univariate analysis of the effects of gender and IgE sensitisation on exhaled NO and flow-independent NO exchange parameters. ANOVA was used when more than two groups were compared and Sheffe's test was used for multiple comparisons between the groups. Linear regression was used to test the correlation

between exhaled NO and cigarette consumption respectively in the univariate analysis of the effect of height, age and FEV_1 on exhaled NO and flow-independent NO exchange parameters. Multiple linear regression was used when analysing the effect of different explanatory variables on exhaled NO and the flow-independent NO exchange parameters. These models always included gender, age, FEV_1 (variables shown to affect $Calv_{NO}$ in our material), height (which affected $FE_{NO\ 0.05}$ and Daw_{NO}) and IgE sensitisation (which affected $FE_{NO\ 0.05}$ and Caw_{NO}). A p-value of < 0.05 was considered statistically significant.

Ethics

All the subjects gave their permission for the utilisation of personal data for the purpose of this study. The study was approved by the Ethics Committee at the Medical Faculty at Uppsala University.

RESULTS

The study population comprised 115 men and 106 women. Their mean age was 43 (range 29-54) years, 35 (15.8%) were current smokers, 75 (33.9%) ex-smokers and 15 (6.8%) passive smokers. The subjects who underwent exhaled NO measurements did not differ from the other participants in the clinical examination in terms of gender, age, smoking history or passive smoking.

Current smoking

Current smokers had significantly lower $FE_{NO\ 0.05}$, Caw_{NO} and $J'aw_{NO}$ values than never-smokers (Table 1). The current smokers differed from ex-smokers only in terms of Caw_{NO} (p=0.02). No correlations could be found between daily cigarette consumption and $FE_{NO\ 0.05}$ or the flow-independent NO exchange parameters.

Table 1 – Exhaled NO (geometric mean (95% CI) in never-, ex- and current smokers

	Never-smokers (n=111)	Ex-smokers (n=75)	p-value*	Current smokers (n=35)	p-value*
$FE_{NO\ 0.05}$ (ppb)	22.8 (20.3-25.7)	17.7 (15.7-20.1)	0.02	14.0 (11.2-17.6)	<0.001
Caw_{NO} (ppb)	126 (114-140)	110 (97-124)	0.26	79.0 (63.0-99.0)	<0.001
Daw_{NO} (mL s^{-1})	9.28 (8.42-10.2)	7.95 (6.99-9.05)	0.18	8.87 (7.05-11.2)	0.92
$Calv_{NO}$ (ppb)	1.32 (1.13-1.54)	1.41 (1.14-1.73)	0.88	0.93 (0.67-1.28)	0.12
$J'aw_{NO}$ (pL s^{-1})	1153 (1011-1315)	858 (745-989)	0.02	688 (529-893)	0.001

* Compared with never-smokers

The association between current smoking and $FE_{NO\ 0.05}$, Caw_{NO} and $J'aw_{NO}$ respectively remained significant when adjusting for gender, age, height, IgE sensitisation and FEV_1

(Table 2). In the multivariable analysis, we also found a significant association between $Calv_{NO}$ and current smoking. In this analysis, height was associated with an increase in $FE_{NO\ 0.05}$ (p=0.04), Daw_{NO} (p=0.03) and $J'aw_{NO}$ values (p=0.03), while IgE sensitisation was associated with an increase in $FE_{NO\ 0.05}$ (p=0.003), Caw_{NO} (p=0.02) and $J'aw_{NO}$ values (p=0.004). Women had higher $Calv_{NO}$ levels than men (p<0.001). Age was positively associated with $Calv_{NO}$ levels (p=0.001). A significant negative association was found between FEV_1 and $Calv_{NO}$ (p<0.001).

Table 2. Association between exhaled NO variables (log-transformed) and smoking history after adjustment for age, gender, height, IgE sensitisation and FEV_1 (effect (95% CI)).

	Ex-smokers (n=72)	p-value*	Current smokers (n=35)	p-value*
$FE_{NO\ 0.05}$ (ppb)	-0.10 (-0.18, -0.02)	0.01	-0.23 (-0.32, -0.13)	<0.001
Caw_{NO} (ppb)	-0.04 (-0.12, 0.03)	0.29	-0.21 (-0.30, -0.11)	<0.001
Daw_{NO} (mL s^{-1})	-0.07 (-0.14, 0.005)	0.07	-0.03 (-0.12, 0.07)	0.56
$Calv_{NO}$ (ppb)	-0.01 (-0.12, 0.10)	0.82	-0.20 (-0.34, -0.06)	0.004
$J'aw_{NO}$ (pL s^{-1})	-0.26 (-0.46, -0.05)	0.01	-0.55 (-0.80, -0.29)	<0.001

* Compared with never-smokers (n=103)

Past smoking

Ex-smokers had significantly lower $FE_{NO\ 0.05}$ values than never-smokers, whereas no significant differences were found for the other NO variables (Table 1). The association between $FE_{NO\ 0.05}$ and past smoking remained significant, after excluding two ex-smokers who had stopped smoking less than one year ago (p=0.03). The association also remained significant after adjusting for gender, age, height, IgE sensitisation and FEV_1 (Table 2).

In ex-smokers, we investigated the effects of smoking-related variables (amount of previous smoking and latency time since quitting smoking respectively), snus consumption and lung function (assessed by FEV_1) on exhaled NO levels. No associations were found between smoking-related variables and exhaled NO levels. Snus consumption in ex-smokers was associated with increased Daw_{NO} ($p=0.04$) in univariate analysis. The association between snus consumption and Caw_{NO} was just above the level of significance ($p=0.06$) (Figure 1). FEV_1 was associated with a reduction in $Calv_{NO}$ ($p=0.01$). The multiple linear regression analysis was used to analyse and confirm these effects in ex-smokers after adjusting for confounding variables. The relationships were maintained and the association between snus consumption and Caw_{NO} in ex-smokers became statistically significant (Table 3).

Table 3. Association between exhaled NO variables (log-transformed) and smoking-related variables in ex-smokers. The effect (95% CI) is estimated after adjustment for the variables in the table and age, gender, height and IgE sensitisation (n=67).

	$FE_{NO\ 0.05}$ (ppb)	Caw_{NO} (ppb)	Daw_{NO} (mL s^{-1})	$Calv_{NO}$ (ppb)	$J'aw_{NO}$ (pL s^{-1})
Latency (per 10 years)	0.04 (-0.04, 0.12)	0.00 (-0.07, 0.08)	0.05 (-0.03, 0.14)	0.01 (-0.11, 0.14)	0.13 (-0.09, 0.34)
Pack years (log-transformed)	0.13 (-0.02, 0.28)	0.06 (-0.08, 0.19)	0.10 (-0.06, 0.26)	-0.10 (-0.32, 0.13)	0.36 (-0.03, 0.75)
Snus consumption	-0.06 (-0.20, 0.08)	-0.19 (-0.32, -0.06)	0.15 (0.004, 0.30)	-0.10 (-0.31, 0.11)	-0.09 (-0.45, 0.26)
FEV_1 (% pred) (10% increase)	-0.03 (-0.06, 0.01)	-0.02 (-0.05, 0.02)	-0.01 (-0.05, 0.03)	-0.07 (-0.12, -0.01)	-0.06 (-0.16, 0.03)

Passive smoking

Non-smokers who were passive smokers had significantly higher $Calv_{NO}$ values than subjects who were not exposed, while there were no significant differences in terms of the other exhaled NO variables (Table 4). The association between passive smoking and increased $Calv_{NO}$ values remained significant after adjustment for age, gender, height, IgE sensitisation and FEV_1 (p=0.008).

Table 4 – Exhaled NO (geometric mean (95% CI) in non-smoking subjects not exposed and exposed to tobacco smoke

	Not exposed to tobacco smoke (n=167)	Exposed to tobacco smoke (n=15)	p-value
$FE_{NO\ 0.05}$ (ppb)	20.7 (18.9-22.7)	20.3 (14.2-29.1)	0.92
Caw_{NO} (ppb)	119 (110-129)	126 (94.2-168)	0.70
Daw_{NO} (mL s^{-1})	8.79 (8.10-9.54)	7.78 (5.63-10.7)	0.40
$Calv_{NO}$ (ppb)	1.29 (1.13-1.48)	2.31 (1.82-2.93)	0.01
$J'aw_{NO}$ (pL s^{-1})	1030 (929-1142)	959 (632-1454)	0.70

DISCUSSION

The main finding in the present study is that current smoking is associated with a reduction in airway and alveolar concentrations of NO. We also found that ex-smokers have lower levels of exhaled NO than never-smokers, which was reflected in a lower total maximum airway NO flux, and that passive smoking was associated with increased alveolar NO concentrations. A surprising and novel finding was that, in ex-smokers, snus consumption was associated with a reduction in airway tissue concentrations of NO and an increase in NO airway transfer factor.

The model used in the present article to determine the NO flow-independent parameters has been validated (19) against the "classical" slope-intercept model (23). The choice of the flow rates and the method used to analyse the data affects the estimation of the NO flow-independent parameters (13). Decreasing the highest flow rate increased the estimated $Calv_{NO}$ in a recent paper that used a linear regression method and FE_{NO} measurements at three flow rates between 100 and 200 mL s^{-1}(24). The choice of the lowest flow rate affects the estimation of Caw_{NO} and Daw_{NO} and theoretically Caw_{NO} would be estimated more accurately by using an as low as possible flow rate as the measured exhaled NO would actually be Caw_{NO} at a flow rate that tends towards 0 mL s^{-1}.

The reduction in airway concentrations of NO in current smokers is in accordance with a previous study from our group (15). A recent study (25) was not able to find differences between smokers and non-smokers in terms of the non-enzymatic production of NO and suggested that the lower levels of exhaled NO in smokers might be due to the down-regulation of enzymatic NO production in the oropharyngeal and bronchial compartment. A negative feedback mechanism caused by the high levels of NO in the cigarette smoke was postulated more than 10 years ago as a possible mechanism (8;9), but not until recently has it

been confirmed in the case of iNOS in lung epithelial cells (8;9). Smoking is associated with reduced levels of BH_4 (10), which might reduce the enzymatic NO production by "uncoupling" NOS, with the resultant production of superoxide instead of NO (26). Superoxide can, in its turn, react with NO to form peroxynitrite. The fact that the NO consumption might be increased in the smokers' airways is also suggested by the increase of NO metabolites in the exhaled breath condensate (12) (27).

In our study, current smoking was also associated with reduced alveolar levels of NO. This result is in accordance with a study by Delclaux *et al.* (14) who measured FE_{NO} at six different flow-rates in the range of 50 to 300 mL s^{-1} and used a least square linear regression in order to obtain $Calv_{NO}$ and $J'aw_{NO}$. Delclaux *et al.* found a trend towards higher values in healthy non-smokers vs. healthy smokers, but contradicted the results of two previous studies from our group where we found either higher $Calv_{NO}$ values in smokers (7) when linear regression was used on FE_{NO} measurements done at five flow rates between 50 and 320 mL s^{-1} or no difference in $Calv_{NO}$ in a study that used the same flow rates and the same model as in the present study (15). One explanation for this apparent discrepancy might be that we adjusted the results for possible confounders, something that was not done in the previous studies. Another possible methodological explanation for this difference might be the fact that this study is based on a general population sample, whereas our previous investigations comprised healthy non-smokers vs healthy smokers. Therriault *et al.* (28) reported that NNK (N-nitrosamine 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone), a component of the cigarette smoke, inhibited alveolar macrophages from producing NO, a finding that might explain the lower alveolar concentrations of NO in smokers. Another possible mechanism could be an increase in the permeability of the respiratory membrane for NO in chronic smokers (29).

We were unable to find a dose-response relationship when studying the number of smoked cigarettes and the levels of exhaled NO. This observation apparently contradicts a previous study by Kharitonov *et al* (3), where a strong correlation between the number of smoked cigarettes and FE_{NO} was found, but this study was conducted in 1995 and used peak exhaled NO concentrations and not the plateau exhaled NO, as in the current recommendations that were followed in the present study. Takahashi *et al*. (30) looked at the end-expiration levels of NO and reported that the levels of exhaled NO were not related to the number of smoked cigarettes.

In our study, ex-smokers had lower levels of $FE_{NO\ 0.05}$ and $J'aw_{NO}$ than never-smokers and this difference remained after adjusting for gender, age, height, IgE sensitisation and FEV_1. This results are in accordance with Robbins *et al.* (6), who reported an increase in FE_{NO}, but still lower levels of mean oral NO than in controls after eight weeks of smoking cessation, but not with a previous study from our group in which we found that four weeks of smoking cessation resulted in an increase in FE_{NO} in the ex-smokers group up to the same level as the healthy non-smoking controls (7). This apparent discrepancy may be due to the fact that some subjects in the smoking cessation group in our previous study had allergic symptoms and therefore had higher "baseline" FE_{NO} levels than the healthy controls who were all non-allergic. Our results point towards a reduction in NO transfer through the apical membrane of the airway epithelial cells, which could be explained by the fact that smoking has been associated with the keratinisation of the epithelial cells, as seen in the case of oral mucosa (31) and tracheal epithelium (32), impeding the NO diffusion.

In our study, we found no association between $FE_{NO\ 0.05}$ and the length of abstinence or the amount of previous smoking in ex-smokers. We did, however, somewhat surprisingly find that snus consumption was associated with a reduction in Caw_{NO} and an increase in Daw_{NO} in ex-smokers. One possible reason for the reduction in Caw_{NO} in snus users may be an increase in the consumption of NO in the airways, possibly due to the transformation of NO to peroxynitrite. This suggestion is supported by the observations of Helen *et al.* (33), who found nicotine-induced peroxidative damage in the lungs, heart and liver of rats. A similar observation was made by Iho *et al.* (34), who looked at the nicotine-stimulated neutrophils and observed that the neutrophilic production of NO was reduced, suggesting that superoxide, produced by nicotine, generates peroxynitrite by reacting with preformed NO.

The other observation that Daw_{NO} was increased in snus consumers might be explained by the higher oral production seen in snus users due to bacterial colonisation. The bacterial colonisation might be explained by the poorer oral hygiene reported in snus consumers, which would create a local environment in the oral mucosa conducive to bacterial growth and colonisation (35). There is evidence that the nicotine concentrations reported in the saliva of snus consumers might have a stimulatory effect on bacterial growth (36). An alternative or complementary explanation could be the high snus content of nitrate (37), which can be transformed in the oral cavity to nitrite by bacterial activity. Nitrite could be subsequently used as substrate to produce NO. The hypothesis that an increase in NO production in the oral cavity will be reflected by Daw_{NO} is indirectly supported by Törnberg *et al.* (38), who observed that, in tracheotomised subjects, the removal of the oropharyngeal compartment led to a significant reduction in Daw_{NO}, without affecting the other flow-independent NO exchange parameters. Törnberg *et al.* have measured exhaled NO at six different flow rates

between 6 and 300 mL s^{-1} and used a non-linear regression method in order to obtain Caw_{NO}, Daw_{NO} and $Calv_{NO}$.

We were not able to demonstrate that reported passive smoking in non-smokers was associated with reduced levels of exhaled NO. This finding is in agreement with some previous studies (39) (40) (41) and is also in partial agreement with Warke *et al.* (5), who used a questionnaire assessment of smoke exposure and were unable to find any effect of smoke exposure on FE_{NO} in non-asthmatic children, although they did find it in asthmatic children. It should be noted that the previous studies were conducted in a population of children and that the only available studies in adults have focused exclusively on the immediate effects of smoking (4) (42). We did find that passive smoking was associated with increased alveolar NO concentrations, but at the present time we have no clear idea of the mechanism behind this observation. One possible explanation might be offered by the reduced permeability of the respiratory membrane seen in subjects exposed to sidestream smoke (43).

The present investigation is one of the first studies to use flow-independent NO exchange parameters in a general population sample. The utilisation of objective markers of tobacco consumption and exposure would have been better, even though there are studies showing a reasonable association between self-reported and objectively measured tobacco use and exposure (44-46).

The use of flow-independent NO exchange parameters may help us to understand the location of the tobacco-induced changes in the airway NO metabolism and exchange. In the present study, both current and past smoking were associated with reduced levels of exhaled NO. In

current smokers, we found reduced NO levels in both the airways and alveoli, while, in the ex-smokers, the total maximum NO flux in the airways was reduced. The association between snus and reduced NO concentrations in the airways and increased NO transfer from the airways warrants further studies.

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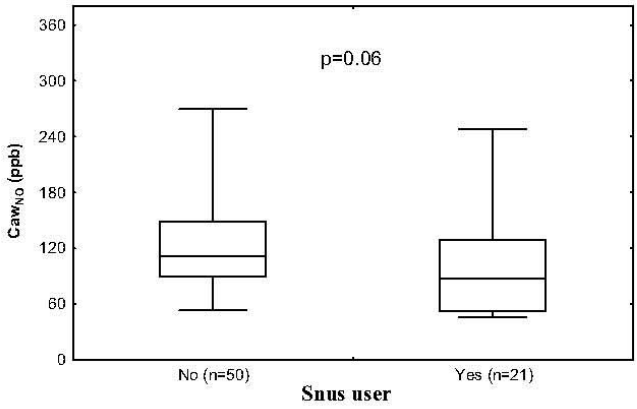
Figure legends

Figure 1. Association between the use of snus in ex-smokers and airway tissue concentrations of NO (Caw_{NO}) and NO airway transfer factor (Daw_{NO}) respectively.

Box-plot explanation: upper horizontal line of box, 75^{th} percentile; lower horizontal line of box, 25^{th} percentile; horizontal bar within box, median; upper horizontal bar outside box, 95^{th} percentile; lower horizontal bar outside box, 5^{th} percentile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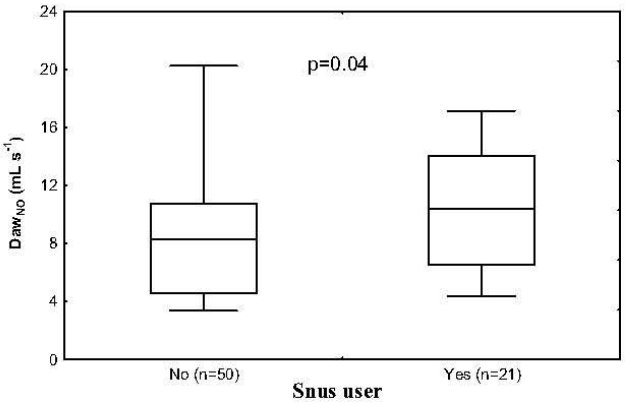
120
60
0
Caw_{NO} (ppb)
No (n=50)
Yes (n=21)
Snus user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24
20
16
12
8
4
0
Daw_{NO} (mL s^{-1})
No (n=50)
Yes (n=21)
Snus user

Reference List

1. Persson MG, Zetterstrom O, Agrenius V, Ihre E, Gustafsson LE. Single-breath nitric oxide measurements in asthmatic patients and smokers. *Lancet* 1994;343:146-147

2. Schilling J, Holzer P, Guggenbach M, Gyurech D, Marathia K, Geroulanos S. Reduced endogenous nitric oxide in the exhaled air of smokers and hypertensives. *Eur Respir J* 1994;7:467-471

3. Kharitonov SA, Robbins RA, Yates D, Keatings V, Barnes PJ. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;152:609-612

4. Yates DH, Breen H, Thomas PS. Passive smoke inhalation decreases exhaled nitric oxide in normal subjects. *Am J Respir Crit Care Med* 2001;164:1043-1046

5. Warke TJ, Mairs V, Fitch PS, Ennis M, Shields MD. Possible association between passive smoking and lower exhaled nitric oxide in asthmatic children. *Arch Environ Health* 2003;58:613-616

6. Robbins RA, Millatmal T, Lassi K, Rennard S, Daughton D. Smoking cessation is associated with an increase in exhaled nitric oxide. *Chest* 1997;112:313-318

7. Hogman M, Holmkvist T, Walinder R, et al. Increased nitric oxide elimination from the airways after smoking cessation. *Clin Sci (Lond)* 2002;103:15-19

8. Assreuy J, Cunha FQ, Liew FY, Moncada S. Feedback inhibition of nitric oxide synthase activity by nitric oxide. *Br J Pharmacol* 1993;108:833-837

9. Hoyt JC, Robbins RA, Habib M, et al. Cigarette smoke decreases inducible nitric oxide synthase in lung epithelial cells. *Exp Lung Res* 2003;29:17-28

10. Higman DJ, Strachan AM, Buttery L, et al. Smoking impairs the activity of endothelial nitric oxide synthase in saphenous vein. *Arterioscler Thromb Vasc Biol* 1996;16:546-552

11. Balint B, Donnelly LE, Hanazawa T, Kharitonov SA, Barnes PJ. Increased nitric oxide metabolites in exhaled breath condensate after exposure to tobacco smoke. *Thorax* 2001;56:456-461

12. Corradi M, Pesci A, Casana R, et al. Nitrate in exhaled breath condensate of patients with different airway diseases. *Nitric Oxide* 2003;8:26-30

13. George SC, Hogman M, Permutt S, Silkoff PE. Modeling pulmonary nitric oxide exchange. *J Appl Physiol* 2004;96:831-839

14. Delclaux C, Mahut B, Zerah-Lancner F, et al. Increased nitric oxide output from alveolar origin during liver cirrhosis versus bronchial source during asthma. *Am J Respir Crit Care Med* 2002;165:332-337

15. Hogman M, Holmkvist T, Wegener T, et al. Extended NO analysis applied to patients with COPD, allergic asthma and allergic rhinitis. *Respir Med* 2002;96:24-30

16. Janson C, Anto J, Burney P, et al. The European Community Respiratory Health Survey: what are the main results so far? European Community Respiratory Health Survey II. *Eur Respir J* 2001;18:598-611

17. The European Community Respiratory Health Survey II. *Eur Respir J* 2002;20:1071-1079

18. Franklin KA, Gislason T, Omenaas E, et al. The influence of active and passive smoking on habitual snoring. *Am J Respir Crit Care Med* 2004;170:799-803

19. Hogman M, Drca N, Ehrstedt C, Merilainen P. Exhaled nitric oxide partitioned into alveolar, lower airways and nasal contributions. *Respir Med* 2000;94:985-991

20. Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 1999;160:2104-2117

21. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;152:1107-1136

22. Standardized lung function testing. Report working party. *Bull Eur Physiopathol Respir* 1983;19 Suppl 5:1-95

23. Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. *J Appl Physiol* 1998;85:653-666

24. Gelb AF, Taylor CF, Nussbaum E, et al. Alveolar and airway sites of nitric oxide inflammation in treated asthma. *Am J Respir Crit Care Med* 2004;170:737-741

25. Marteus H, Mavropoulos A, Palm JP, Ulfgren AK, Bergstrom J, Alving K. Nitric oxide formation in the oropharyngeal tract: possible influence of cigarette smoking. *Nitric Oxide* 2004;11:247-255

26. Wever RM, van DT, van Rijn HJ, de GF, Rabelink TJ. Tetrahydrobiopterin regulates superoxide and nitric oxide generation by recombinant endothelial nitric oxide synthase. *Biochem Biophys Res Commun* 1997;237:340-344

27. Garey KW, Neuhauser MM, Robbins RA, Danziger LH, Rubinstein I. Markers of inflammation in exhaled breath condensate of young healthy smokers. *Chest* 2004;125:22-26

28. Therriault MJ, Proulx LI, Castonguay A, Bissonnette EY. Immunomodulatory effects of the tobacco-specific carcinogen, NNK, on alveolar macrophages. *Clin Exp Immunol* 2003;132:232-238

29. Jones JG, Minty BD, Lawler P, Hulands G, Crawley JC, Veall N. Increased alveolar epithelial permeability in cigarette smokers. *Lancet* 1980;1:66-68

30. Takahashi Y, Kobayashi H, Takahashi Y, Mitsufuji H, Tomita T. [A standard maneuver to measure the concentration of exhaled nitric oxide]. *Nihon Kyobu Shikkan Gakkai Zasshi* 1997;35:596-601

31. Mosadomi A, Shklar G, Loftus ER, Chauncey HH. Effects of tobacco smoking and age on the keratinization of palatal mucosa: a cytologic study. *Oral Surg Oral Med Oral Pathol* 1978;46:413-417

32. Rutten AA, Bruyntjes JP, Ramaekers FC. Effect of cigarette smoke condensate and vitamin A depletion on keratin expression patterns in cultured hamster tracheal epithelium. An immunohistomorphological study using monoclonal antibodies to keratins. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1988;56:111-117

33. Helen A, Krishnakumar K, Vijayammal PL, Augusti KT. Antioxidant effect of onion oil (Allium cepa. Linn) on the damages induced by nicotine in rats as compared to alpha-tocopherol. *Toxicol Lett* 2000;116:61-68

34. Iho S, Tanaka Y, Takauji R, et al. Nicotine induces human neutrophils to produce IL-8 through the generation of peroxynitrite and subsequent activation of NF-kappaB. *J Leukoc Biol* 2003;74:942-951

35. Rubinstein I, Pedersen GW. Bacillus species are present in chewing tobacco sold in the United States and evoke plasma exudation from the oral mucosa. *Clin Diagn Lab Immunol* 2002;9:1057-1060

36. Keene K, Johnson RB. The effect of nicotine on growth of Streptococcus mutans. *Miss Dent Assoc J* 1999;55:38-39

37. Brunnemann KD, Qi J, Hoffmann D. Chemical profile of two types of oral snuff tobacco. *Food Chem Toxicol* 2002;40:1699-1703

38. Tornberg DC, Bjorne H, Lundberg JO, Weitzberg E. Multiple single-breath measurements of nitric oxide in the intubated patient. *Am J Respir Crit Care Med* 2003;168:1210-1215

39. Franklin PJ, Taplin R, Stick SM. A community study of exhaled nitric oxide in healthy children. *Am J Respir Crit Care Med* 1999;159:69-73

40. Barreto M, Villa MP, Martella S, et al. Exhaled nitric oxide in asthmatic and non-asthmatic children: influence of type of allergen sensitization and exposure to tobacco smoke. *Pediatr Allergy Immunol* 2001;12:247-256

41. Janson C, Kalm-Stephens P, Foucard T, Norback D, Alving K, Nordvall SL. Exhaled nitric oxide levels in school children in relation to IgE sensitisation and window pane condensation. *Respir Med* 2005;99:1015-1021

42. Maniscalco M, Di M, V, Farinaro E, Carratu L, Sofia M. Transient decrease of exhaled nitric oxide after acute exposure to passive smoke in healthy subjects. *Arch Environ Health* 2002;57:437-440

43. Yates DH, Havill K, Thompson MM, Rittano AB, Chu J, Glanville AR. Sidestream smoke inhalation decreases respiratory clearance of 99mTc-DTPA acutely. *Aust N Z J Med* 1996;26:513-518

44. Cabrera dL, Almeida GD, Perez ML, et al. [Cigarette smoking among adolescents. Estimated prevalence by a questionnaire and serum cotinine]. *Gac Sanit* 1999;13:270-274

45. McDonald SP, Maguire GP, Hoy WE. Validation of self-reported cigarette smoking in a remote Australian Aboriginal community. *Aust N Z J Public Health* 2003;27:57-60

46. Riboli E, Preston-Martin S, Saracci R, et al. Exposure of nonsmoking women to environmental tobacco smoke: a 10-country collaborative study. *Cancer Causes Control* 1990;1:243-252