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Effect of smoking on exhaled nitric oxide and flow-independent nitric oxide exchange parameters

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ABSTRACT: 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 production or exchange of NO in different compartments of the airways.

This study comprised 221 adult subjects from the European Community Respiratory Health Survey II, who were investigated in terms of their exhaled NO, lung function, immunoglobulin E sensitisation and smoking habits. The following parameters were determined using extended NO analysis: airway tissue nitric oxide concentration ($C_{aw,NO}$), airway transfer factor (or diffusing capacity) for nitric oxide ($D_{aw,NO}$), alveolar nitric oxide concentration ($C_{A,NO}$) and fractional exhaled nitric oxide concentration at a flow rate of 50 mL·s^{-1} ($F_{eNO,0.05}$). Maximum total airway nitric oxide flux ($J'_{aw,NO}$) was calculated from $D_{aw,NO}(C_{aw,NO}–C_{A,NO})$.

Current smokers (n=35) exhibited lower (geometric mean) $F_{eNO,0.05}$ (14.0 *versus* 22.8 ppb), $C_{aw,NO}$ (79.0 *versus* 126 ppb) and $J'_{aw,NO}$ (688 *versus* 1,153 pL·s^{-1}) than never-smokers (n=111). Ex-smokers (n=75) were characterised by lower $F_{eNO,0.05}$ (17.7 *versus* 22.8 ppb) and $J_{aw,NO}$ (858 *versus* 1,153 pL·s^{-1}) than never-smokers. These relationships were maintained after adjusting for potential confounders (sex, age, height, immunoglobulin E sensitisation and forced expiratory volume in one second), and, in this analysis, a negative association was found between current smoking and $C_{A,NO}$. Snus (oral moist snuff) consumption (n=21) in ex-smokers was associated with an increase in $D_{aw,NO}$ and a reduction in $C_{aw,NO}$, after adjusting for potential confounders. Passive smoking was associated with a higher $C_{A,NO}$.

Using extended nitric oxide analysis, it was possible to attribute the reduction in exhaled nitric oxide levels seen in ex- and current smokers to a lower total airway nitric oxide flux in ex-smokers and reduced airway and alveolar nitric oxide concentrations in current smokers. The association between snus (oral tobacco) use and reduced nitric oxide concentrations in the airways and increased nitric oxide transfer from the airways warrants further studies.

KEYWORDS: Exhaled nitric oxide, extended nitric oxide analysis, oral tobacco, smoking

A reduction in exhaled nitric oxide (NO) levels was first observed in smokers in the early 1990s [1, 2], and this effect was found after both acute and chronic exposure to smoking [3]. Passive smoking has also been found to reduce 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, which could lead to downregulation of NO synthase (NOS) in the lungs [8, 9], an inadequate supply of cofactors necessary for NO production, such as tetrahydrobiopterin [10], and an increase in the breakdown of NO [11, 12].

By modelling NO exchange dynamics, it is possible to obtain 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 on this topic has been published recently [13]. The few studies investigating the effects of smoking on flow-independent NO exchange

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parameters indicate that smoking is related to a lower maximum total airway nitric oxide flux ($J'_{aw,NO}$) [7, 14] and to a lower mean airway tissue nitric oxide concentration ($C_{aw,NO}$) [15]. There is, however, very limited information about the dose–response relationship and the effect of past 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 the present study were participants in a follow-up of the European Community Respiratory Health Survey (ECRHS), which was performed in Uppsala during 1990–1991 [16]. Of the 622 subjects in the random sample of the ECRHS, 517 were reinvestigated 9 yrs later (1999–2000) in the ECRHS II [17]. The majority of the subjects who were reinvestigated (n=368) were seen at the hospital for a clinical examination, whereas the remaining 149 subjects participated only in a telephone survey, usually because they had moved outside the study area between the two surveys.

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

Questionnaires

The ECHRS II main questionnaire [17] was used to obtain information about symptoms, diagnoses, smoking history and habits. Additionally, information about snus (oral moist snuff (tobacco)) consumption was obtained from the sleep questionnaire included in the Respiratory Health in Northern Europe study [18].

Measurements of exhaled NO

NO measurements were performed according to American Thoracic Society (ATS) recommendations, apart from the use of three additional flow rates (5, 100 and 500 $mL \cdot s^{-1}$) 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 (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, Lidingö, Sweden) and the zero was set by feeding synthetic air (AGA) into a 2-L canister filled with Purafil II chemisorbent with purakol (Lindair, Ljusne, Sweden). The flow sensor was calibrated in the range 0–0.6 $L \cdot s^{-1}$ (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 ranged 5–20 cmH_2O in order to exclude a NO contribution from the nasal cavity. The mean value from 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 fractional exhaled NO concentration (F_{eNO}) obtained at three different flow rates (5, 100 and 500 $mL \cdot s^{-1}$) and an iterative algorithm, it calculates the three flow-independent NO exchange parameters confined to the two compartments, the conducting airways, which are characterised by the $C_{aw,NO}$ and airway transfer factor (or diffusing capacity) for nitric oxide ($D_{aw,NO}$), and the alveoli, characterised by the alveolar nitric oxide concentration ($C_{A,NO}$). A fourth variable, $J'_{aw,NO}$, was also used. $J'_{aw,NO}$ is calculated from $D_{aw,NO}(C_{aw,NO}–C_{A,NO})$. The reason for including $J'_{aw,NO}$ in the present study was that it provides a global airway compartment description. The F_{eNO} at a flow rate of 50 mL s^{-1} ($F_{eNO,0.05}$) was used as a measure of overall exhaled NO concentration. It was decided to use the $F_{eNO,0.05}$ 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 (SensorMedics 2130; SensorMedics, Anaheim, CA, USA). Up to five technically acceptable blows were measured. ATS recommendations were followed [21]. The predicted values for FEV_1 were calculated on the basis of European Coal and Steel Union reference values [22].

Immunoglobulin E sensitisation

Blood samples were collected for the measurement of total and specific serum immunoglobulin (Ig)E using the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). Specific IgE directed against *Dermatophagoides pteronyssinus*, cat, timothy grass and *Cladosporium herbarum* were measured. The detection of a specific IgE concentration of >0.35 $kU \cdot L^{-1}$ was used as the 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 on starting, amount smoked currently, whether they had stopped or cut down, and the amount smoked previously. Based on this information, the subjects were classified as never-smokers, ex-smokers and smokers. The mean number of cigarettes smoked per day was used to quantify exposure in current smokers. Lifetime exposure to smoking was calculated in pack-yrs. 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 passive smokers.

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

Statistical methods

NO values, mean daily cigarette consumption and pack-yrs of consumption were log-transformed before analysis. An unpaired t-test was used for comparisons between ex-smokers with and without snus consumption and in univariate analysis of the effects of sex and IgE sensitisation on exhaled NO and flow-independent NO exchange parameters. ANOVA was used when more than two groups were compared and Scheffé'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 FEV1 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 sex, age and FEV1 (variables shown to affect $C_{A,NO}$ in the present study), height (which affected $F_{eNO,0.05}$ and $D_{aw,NO}$), and IgE sensitisation (which affected $F_{eNO,0.05}$ and $C_{aw,NO}$). A p-value of <0.05 was considered significant.

Ethics

All of the subjects gave their permission for the utilisation of their personal data for the purposes of the present study. The study was approved by the Ethics Committee of the Medical Faculty of Uppsala University (Uppsala, Sweden).

RESULTS

The study population comprised 115 males and 106 females. Their mean age was 43 yrs (range 29–54 yrs); 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 undergoing clinical examination in terms of sex, age, smoking history or passive smoking.

Current smoking

Current smokers exhibited significantly lower $F_{eNO,0.05}$, $C_{aw,NO}$ and $J'_{aw,NO}$ than never-smokers (table 1). The current smokers differed from ex-smokers only in terms of $C_{aw,NO}$ ($p=0.02$). No correlations could be found between daily cigarette consumption and $F_{eNO,0.05}$ or the flow-independent NO exchange parameters.

The association between current smoking and $F_{eNO,0.05}$, $C_{aw,NO}$ and $J'_{aw,NO}$, respectively, remained significant when adjusting for sex, age, height, IgE sensitisation and FEV1 (table 2). In the multivariable analysis, a significant association was also found between $C_{A,NO}$ and current smoking. In this analysis, height was associated with an increase in $F_{eNO,0.05}$ ($p=0.04$), $D_{aw,NO}$ ($p=0.03$) and $J'_{aw,NO}$ ($p=0.03$), whereas IgE sensitisation was associated with an increase in $F_{eNO,0.05}$ ($p=0.003$), $C_{aw,NO}$ ($p=0.02$) and $J'_{aw,NO}$ ($p=0.004$). Females exhibited higher $C_{A,NO}$ than males ($p<0.001$). Age was positively associated with $C_{A,NO}$ ($p=0.001$). A significant negative association was found between FEV1 and $C_{A,NO}$ ($p<0.001$).

Past smoking

Ex-smokers showed significantly lower $F_{eNO,0.05}$ than never-smokers, whereas no significant differences were found for the

TABLE 2 Association between exhaled nitric oxide (NO) variables and smoking history[#]

	Ex-smokers	p-value[¶]	Current smokers	p-value[¶]
Subjects n	72		35	
$F_{eNO,0.05}$ ppb	-0.10 (-0.18– -0.02)	0.01	-0.23 (-0.32– -0.13)	<0.001
$C_{aw,NO}$ ppb	-0.04 (-0.12–0.03)	0.29	-0.21 (-0.30– -0.11)	<0.001
$D_{aw,NO}$ mL·s^{-1}	-0.07 (-0.14–0.005)	0.07	-0.03 (-0.12–0.07)	0.56
$C_{A,NO}$ 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

Data are presented as effect estimate (95% confidence interval), unless otherwise stated. Nitric oxide variables are log-transformed. The effect estimate is the regression coefficient; however, it is difficult to transform this into a percentage increase/decrease since comparisons have been made between transformed and non-transformed data. $F_{eNO,0.05}$: fractional exhaled nitric oxide concentration at a flow rate of 50 mL·s^{-1}; $C_{aw,NO}$: airway tissue nitric oxide concentration; $D_{aw,NO}$: airway transfer factor (or diffusing capacity) for nitric oxide; $C_{A,NO}$: alveolar nitric oxide concentration; $J'_{aw,NO}$: maximum total airway nitric oxide flux. [#]: after adjustment for age, sex, height, immunoglobulin E sensitisation and forced expiratory volume in one second; [¶]: *versus* never-smokers (n=103).

TABLE 1 Exhaled nitric oxide variables in never-, ex- and current smokers

	Never-smokers	Ex-smokers	p-value[#]	Current smokers	p-value[#]
Subjects n	111	75		35	
$F_{eNO,0.05}$ ppb	22.8 (20.3–25.7)	17.7 (15.7–20.1)	0.02	14.0 (11.2–17.6)	<0.001
$C_{aw,NO}$ ppb	126 (114–140)	110 (97–124)	0.26	79.0 (63.0–99.0)	<0.001
$D_{aw,NO}$ 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
$C_{A,NO}$ 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

Data are presented as geometric mean (95% confidence interval), unless otherwise stated. $F_{eNO,0.05}$: fractional exhaled nitric oxide concentration at a flow rate of 50 mL·s^{-1}; $C_{aw,NO}$: airway tissue nitric oxide concentration; $D_{aw,NO}$: airway transfer factor (or diffusing capacity) for nitric oxide; $C_{A,NO}$: alveolar nitric oxide concentration; $J'_{aw,NO}$: maximum total airway nitric oxide flux. [#]: *versus* never-smokers.

other NO variables (table 1). The association between $F_{ENO,0.05}$ and past smoking remained significant after excluding two ex-smokers who had stopped smoking <1 yr previously (p=0.03). The association also remained significant after adjusting for sex, age, height, IgE sensitisation and FEV1 (table 2).

In ex-smokers, the effects of smoking-related variables (amount previously smoked and latency respectively), snus consumption and lung function (assessed by FEV1) on exhaled NO levels were investigated. No associations were found between smoking-related variables and exhaled NO levels. Snus consumption in ex-smokers was associated with increased $D_{aw,NO}$ (p=0.04) on univariate analysis. The association between snus consumption and $C_{aw,NO}$ was just above the level of significance (p=0.06; fig. 1). FEV1 was associated with a reduction in $C_{A,NO}$ (p=0.01). 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 $C_{aw,NO}$ in ex-smokers became significant (table 3).

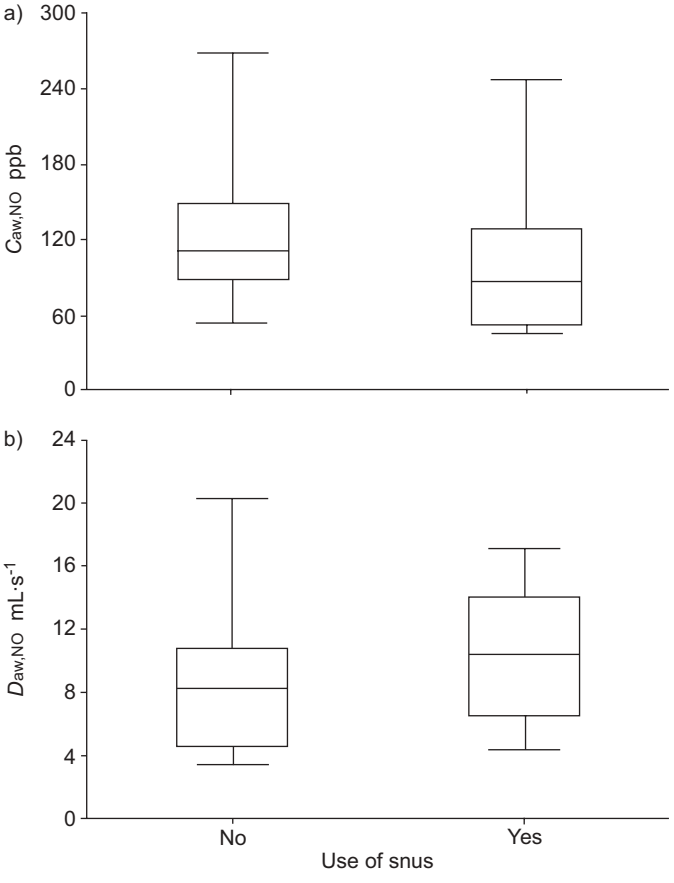

FIGURE 1. Boxplot showing association between use of snus (oral moist snuff; 21 users and 50 nonusers) in ex-smokers and: a) airway tissue nitric oxide concentration ($C_{aw,NO}$; p=0.06); and b) airway transfer factor (or diffusing capacity) for nitric oxide ($D_{aw,NO}$; p=0.04). Boxes represent median and interquartile range; horizontal bars represent 5th–95th percentile range.

Passive smoking

Nonsmokers who were passive smokers exhibited significantly higher $C_{A,NO}$ than subjects who were not exposed, whereas there were no significant differences in terms of the other exhaled NO variables (table 4). The association between passive smoking and increased $C_{A,NO}$ remained significant after adjustment for age, sex, height, IgE sensitisation and FEV1 (p=0.008).

DISCUSSION

The main finding of the present study is that current smoking is associated with a reduction in $C_{aw,NO}$ and $C_{A,NO}$. It was also found that ex-smokers exhibited lower levels of exhaled NO than never-smokers, which was reflected in a lower $J'_{aw,NO}$, and that passive smoking was associated with increased $C_{A,NO}$. A surprising and novel finding was that, in ex-smokers, snus consumption was associated with a reduction in $C_{aw,NO}$ and an increase in $D_{aw,NO}$.

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 flow rates and method used to analyse the data affects the estimation of NO flow-independent parameters [13]. Decreasing the highest flow rate increased the estimated $C_{A,NO}$ in a recent article that used a linear regression method and F_{ENO} measurements at three flow rates ranging 100–200 mL·s^{-1} [24]. The choice of lowest flow rate affects the estimation of $C_{aw,NO}$ and $D_{aw,NO}$, and, theoretically, $C_{aw,NO}$ would be estimated more accurately by using as low as possible a flow rate, since the measured exhaled NO would be $C_{aw,NO}$ at a flow rate that tends towards 0 mL·s^{-1}.

The reduction in $C_{aw,NO}$ in current smokers is in accordance with a previous study [15]. A recent study [25] was not able to demonstrate differences between smokers and nonsmokers in terms of the nonenzymatic production of NO and suggested that the lower levels of exhaled NO in smokers might be due to the downregulation of enzymatic NO production in the oropharyngeal and bronchial compartment. A negative feedback mechanism caused by the high levels of NO in cigarette smoke was postulated, in the early 1990s, as a possible mechanism [8], but this was only confirmed in 2003, in the case of inducible NOS in lung epithelial cells [9]. Smoking is associated with reduced levels of tetrahydrobiopterin [10], which might reduce enzymatic NO production by uncoupling NOS, with resultant production of superoxide instead of NO [26]. Superoxide can, in turn, react with NO to form peroxynitrite. The fact that NO consumption might be increased in smokers' airways is also suggested by the increase in NO metabolites in exhaled breath condensate [12, 27].

In the present study, current smoking was also associated with reduced $C_{A,NO}$. This result is in accordance with a study of DELCLAUX *et al.* [14], who measured F_{ENO} at six different flow rates, ranging 50–300 mL·s^{-1}, and used least-squares linear regression in order to obtain $C_{A,NO}$ and $J'_{aw,NO}$. DELCLAUX *et al.* [14] found a trend towards higher values in healthy nonsmokers *versus* healthy smokers, but contradicted the results of two previous studies demonstrating either higher $C_{A,NO}$ in smokers [7], when linear regression was used on F_{ENO} measurements performed at five flow rates, ranging 50–320 mL·s^{-1}, or no

TABLE 3 Association between exhaled nitric oxide variables and smoking-related variables in ex-smokers[#]

	$F_{ENO,0.05}$ ppb	$C_{aw,NO}$ ppb	$D_{aw,NO}$ mL·s^{-1}	$C_{A,NO}$ ppb	$J'_{aw,NO}$ pL·s^{-1}
Latency per 10 yrs	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)
Cigarette consumption pack-yrs	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)
FEV1 % pred[§]	-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)

Data are presented as effect estimate (95% confidence interval), unless otherwise stated. Nitric oxide variables and cigarette consumption are log-transformed. The effect estimate is the regression coefficient; however, it is difficult to transform this into a percentage increase/decrease since comparisons have been made between transformed and non-transformed data. $F_{ENO,0.05}$: fractional exhaled nitric oxide concentration at a flow rate of 50 mL·s^{-1}; $C_{aw,NO}$: airway tissue nitric oxide concentration; $D_{aw,NO}$: airway transfer factor (or diffusing capacity) for nitric oxide; $C_{A,NO}$: alveolar nitric oxide concentration; $J'_{aw,NO}$: maximum total airway nitric oxide flux; FEV1: forced expiratory volume in one second. [#]: after adjustment for the variables in the table and age, sex, height and immunoglobulin E sensitisation (n=67); [¶]: oral moist snuff; [+]: yes/no; [§]: 10% increase in value.

difference in $C_{A,NO}$, in a study that used the same flow rates and model as the present study [15]. One explanation for this apparent discrepancy might be that the results were adjusted for possible confounders, something that was not undertaken in the previous studies. Another possible methodological explanation for this difference might be the fact that the present study is based on a general population sample, whereas the previous investigations comprised healthy nonsmokers *versus* healthy smokers. THERRIAULT *et al.* [28] reported that the *N*-nitrosamine 4-(*N*-methylnitrosamino)-1-(3-pyridyl)-1-butanone, a component of cigarette smoke, inhibited alveolar macrophages from producing NO, a finding that might explain the lower $C_{A,NO}$ in smokers. Another possible mechanism could be an increase in the permeability of the respiratory membrane for NO in chronic smokers [29].

It was not possible to find a dose–response relationship when studying the number of cigarettes smoked and levels of exhaled NO. This observation apparently contradicts a previous study of KHARITONOV *et al.* [3], in which a strong correlation between the number of cigarettes smoked and F_{ENO} was found, but this study was conducted in 1995 and used peak and not plateau exhaled NO concentrations, as in the current recommendations, which were followed in the present study. TAKAHASHI *et al.* [30] looked at end-expiratory levels of NO and reported that levels of exhaled NO were not related to the number of cigarettes smoked.

TABLE 4 Exhaled nitric oxide in nonsmoking subjects not exposed and exposed to tobacco smoke

	Not exposed	Exposed	p-value
Subjects n	167	15	
$F_{ENO,0.05}$ ppb	20.7 (18.9–22.7)	20.3 (14.2–29.1)	0.92
$C_{aw,NO}$ ppb	119 (110–129)	126 (94.2–168)	0.70
$D_{aw,NO}$ mL·s^{-1}	8.79 (8.10–9.54)	7.78 (5.63–10.7)	0.40
$C_{A,NO}$ 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

Data are presented as geometric mean (95% confidence interval), unless otherwise stated. $F_{ENO,0.05}$: fractional exhaled nitric oxide concentration at a flow rate of 50 mL·s^{-1}; $C_{aw,NO}$: airway tissue nitric oxide concentration; $D_{aw,NO}$: airway transfer factor (or diffusing capacity) for nitric oxide; $C_{A,NO}$: alveolar nitric oxide concentration; $J'_{aw,NO}$: maximum total airway nitric oxide flux.

In the present study, ex-smokers exhibited lower $F_{ENO,0.05}$ and $J'_{aw,NO}$ than never-smokers, and this difference remained after adjusting for sex, age, height, IgE sensitisation and FEV1. These results are in accordance with those of ROBBINS *et al.* [6], who reported an increase in F_{ENO} but even lower levels of mean oral NO than in controls after 8 weeks of smoking cessation. The results are not in accordance with a previous study in which it was found that 4 weeks of smoking cessation resulted in an increase in F_{ENO} in the ex-smoker group up to the same level as the healthy nonsmoking controls [7]. This apparent discrepancy may be due to the fact that some of the subjects in the smoking cessation group in the previous study showed allergic symptoms and therefore higher baseline F_{ENO} than the healthy controls, who were all nonallergic. The present 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 epithelial cells, as seen for oral mucosa [31] and tracheal epithelium [32], impeding NO diffusion.

In the present study, no association was found between $F_{ENO,0.05}$ and latency or amount of previous smoking in ex-smokers. However, somewhat surprisingly, it was found that snus consumption was associated with a reduction in $C_{aw,NO}$ and an increase in $D_{aw,NO}$ in ex-smokers. One possible reason for the reduction in $C_{aw,NO}$ 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 nicotine-stimulated neutrophils and noted that neutrophilic production of NO was reduced, suggesting that superoxide, produced by nicotine, generates peroxynitrite by reacting with preformed NO.

The other observation, which was that $D_{aw,NO}$ 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 nitrate content of snus [37], which can be transformed in the oral cavity to nitrite by bacterial activity. Nitrite could subsequently be used as substrate to produce NO. The hypothesis that increased NO production in the oral cavity is reflected in the $D_{aw,NO}$ was 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 $D_{aw,NO}$, without affecting the other flow-independent NO exchange parameters. TÖRNBERG *et al.* [38] measured exhaled NO at six different flow rates ranging 6–300 $mL \cdot s^{-1}$ and used a nonlinear regression method to obtain $C_{aw,NO}$, $D_{aw,NO}$ and $C_{A,NO}$.

It was not possible to demonstrate that reported passive smoking in nonsmokers was associated with reduced levels of exhaled NO. This finding is in agreement with some previous studies [39–41] and also in partial agreement with the study of WARKE *et al.* [5], which used questionnaire assessment of smoke exposure and was unable to show any effect of smoke exposure on F_{eNO} in nonasthmatic children, although it was found 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]. It was found that passive smoking was associated with increased $C_{A,NO}$, but, at the present time, the current authors have no clear idea as to 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 investigate 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 nitric oxide exchange parameters may aid understanding of the location of tobacco-induced changes in airway nitric oxide metabolism and exchange. In the present study, both current and past smoking were associated with reduced levels of exhaled nitric oxide. In current smokers, reduced nitric oxide levels were found in both the airways and alveoli, whereas, in ex-smokers, the maximum total airway nitric oxide flux was reduced. The association between snus (oral moist snuff) and reduced nitric oxide concentrations in the airways and increased nitric oxide transfer from the airways warrants further studies.

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