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## Influence of mouthwashes on extended exhaled nitric oxide ( $F_{\text{ENO}}$ ) analysis

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### ABSTRACT

Fractional exhaled nitric oxide ( $F_{\text{ENO}}$ ) is used to assess eosinophilic inflammation of the airways.  $F_{\text{ENO}}$  values are influenced by the expiratory flow rate and orally produced NO. We measured  $F_{\text{ENO}}$  at four different expiratory flow levels after two different mouthwashes: tap water and carbonated water. Further, we compared the alveolar NO concentration ( $C_{\text{ANO}}$ ), maximum airway NO flux ( $J'_{\text{awNO}}$ ) and airway NO diffusion ( $D_{\text{awNO}}$ ) after these two mouthwashes.  $F_{\text{ENO}}$  was measured in 30 volunteers (healthy or asthmatic) with a chemiluminescence NO-analyser at flow rates of 30, 50, 100 and 300 mL/s. A mouthwash was performed before the measurement at every flow rate. The carbonated water mouthwash significantly reduced  $F_{\text{ENO}}$  compared to the tap water mouthwash at all expiratory flows: 50 mL/s ( $p < .001$ ), 30 mL/s ( $p = .001$ ), 100 mL/s ( $p < .001$ ) and 300 mL/s ( $p = .004$ ).  $J'_{\text{awNO}}$  was also significantly reduced ( $p = .017$ ), however, there were no significant differences in  $C_{\text{ANO}}$  and  $D_{\text{awNO}}$ . In conclusion, a carbonated water mouthwash can significantly reduce oropharyngeal NO compared to a tap water mouthwash at expiratory flows of 30–300 mL/s without affecting the  $C_{\text{ANO}}$  and  $D_{\text{awNO}}$ . Therefore, mouthwashes need to be taken into account when comparing  $F_{\text{ENO}}$  results.

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### KEYWORDS

Nitric oxide (NO); alveolar NO concentration ( $C_{\text{ANO}}$ ); asthma; fractional exhaled nitric oxide ( $F_{\text{ENO}}$ ); maximum airway NO flux ( $J'_{\text{awNO}}$ ); mouthwashes; carbonated water; inflammation; respiratory system; exhalation; airway obstruction; oropharynx

### Introduction

Eosinophilic inflammation of the bronchial epithelium is a chronic process often leading to bronchial hyperreactivity and airway obstruction. During inflammation, fractional exhaled nitric oxide ( $F_{\text{ENO}}$ ) is mainly produced in the airway epithelium, catalysed by inducible NO synthase (NOS2) [1]. In clinical practice, assessing  $F_{\text{ENO}}$  facilitates asthma diagnosis [2].

$F_{\text{ENO}}$  is a combination of nitric oxide (NO) originating in the lung periphery, the bronchioli, the bronchi and the central large airways, and  $F_{\text{ENO}}$  depends highly on the expiratory flow rates. NO dynamics in the lung periphery determine to a large extent  $F_{\text{ENO}}$  measured at higher flow rates, while mainly bronchial NO dynamics determine  $F_{\text{ENO}}$  measured at slower flow rates [3,4].  $F_{\text{ENO}}$  measurement has been previously recommended at the expiratory flow rate of 50 mL/s [5,6]. There is evidence that  $F_{\text{ENO}}$  values over 50 ppb (>35 ppb in children) measured at this flow indicate eosinophilic airway inflammation and the cut-off can be applied to detect asthma in symptomatic individuals [7,8].

$F_{\text{ENO}}$  measurement at multiple flows are used to estimate the anatomical origin of NO in exhaled air [9–12]. A simple two compartment model of the airways has been adopted [6] to define the alveolar NO concentration ( $C_{\text{ANO}}$ ), maximum airway NO flux ( $J'_{\text{awNO}}$ ) and airway NO diffusion

( $D_{\text{awNO}}$ ) [13]. These estimates have provided extended information in subjects with asthma [12–15].



In healthy subjects, a fraction of  $F_{\text{ENO}}$  seems to originate in the upper airways and oropharynx, partly due to bacterial production of NO and dietary intake of nitrate [16–21].

Rinsing the oral cavity with carbonated water lowers  $F_{\text{ENO}}$  levels significantly in healthy and asthmatic subjects [22,23]. Although the ATS/ERS guidelines suggest that a mouthwash before  $F_{\text{ENO}}$  measurements may reduce oral  $F_{\text{ENO}}$ , it is not part of the standardized clinical procedure [5], but only recommended in physiological research [6]. However, rinsing of the mouth with carbonated water has been routinely adopted in our laboratory at the Helsinki University Hospital prior to  $F_{\text{ENO}}$  measurements since 1995.

The aim of this study was to compare the effects of a carbonated water mouthwash and a tap water mouthwash on  $F_{\text{ENO}}$  at different flow rates, as well to analyse the effects of the mouthwashes on  $C_{\text{ANO}}$ ,  $J'_{\text{awNO}}$  and  $D_{\text{awNO}}$ . Furthermore, we aimed to investigate the repeatability of the  $F_{\text{ENO}}$  measurements.

### Methods

We recruited 30 volunteers aged 16–68 years, either health-care workers ( $n = 21$ ) or patients ( $n = 9$ ). The patients

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enrolled have had asthmatic symptoms and were previously referred for  $F_{\text{ENO}}$  testing, either to the Laboratory of Clinical Physiology at the University Hospital in Helsinki or the Skin and Allergy Hospital in Helsinki. Healthcare workers who volunteered were included without further selection. None of the subjects were excluded. All participants were asked to fill a questionnaire including questions regarding smoking habits, asthma, allergic rhinitis and COPD.

Nine participants reported having allergic rhinitis, six asthma and one COPD. Two participants smoked regularly, one less than five cigarettes a day, the other from 5 to 15 a day. From available medical records, we searched for respiratory or systemic diseases that could influence  $F_{\text{ENO}}$  values, reason for referral to  $F_{\text{ENO}}$  testing and current medication. Nine participants had a chronic respiratory disease or respiratory symptoms. Among these were four patients with asthma, three with respiratory symptoms due to building dampness but low or negative bronchial hyperreactivity, one with eosinophilic bronchitis and another with Sjögren's syndrome. The patient with Sjögren's syndrome had no interstitial lung disease, previously ruled out through a high resolution computed tomography. Six participants had prescriptions for short acting  $\beta_2$ -agonists, four used inhaled corticosteroids regularly, two used antihistamines and one used leukotriene receptor antagonists. Spirometric data were available in 25 subjects. None of these subjects had current bronchodilator reversibility (defined as increase in FEV1 or FVC over 12% and 200 mL) [24]. Demographic, anthropometric and spirometric data are summarised in Table 1.

The  $F_{\text{ENO}}$  measurements were made either at the Skin and Allergy Hospital or at the Finnish Institute of Occupational Health, both in Helsinki. The NO analysers used were chemiluminescence CLD 88 sp and the devices were EXHALIZER<sup>®</sup>s D with SPIROWARE<sup>®</sup> software from Eco Medics AG (Dürnten, Switzerland) and calibrated according to

the manufacturer's instructions by using certified span gas (AGA Gas BV, Amsterdam, Netherlands) and NO free air by using a zero-air filter (DENOX 88 unit). The inspired gas was NO free (<5 ppb, maximum recorded fractional inspired  $F_{\text{ino}}$  was 3.1 ppb). Before the measurements, the ultrasonic flow sensor was calibrated with a calibration syringe (Hans Rudolph Inc., Shawnee, KS).  $F_{\text{ENO}}$  measurements for each subject were performed during two consecutive days. The flow-chart in Figure 1 visualizes the order of the procedures. The measurements were made at four different expiratory flow rates  $V'$  (30, 50, 100 and 300 mL/s). The sequence of the flow rates was kept the same, starting with a 50 mL/s flow rate, followed by 30, 100 and 300 mL/s. The mouthwash procedure was defined as rinsing the oral cavity for 30–60 seconds with 100 mL of tap water or carbonated water. On the first day, the subjects performed a mouthwash with tap water before the measurements at the first flow level, and then repeated the mouthwash with tap water before measuring at every flow level. After reaching the highest flow level, i.e. 300 mL/s, there was a 15 min pause before starting a new array. After this time interval, all measurements were repeated, but 100 mL of carbonated water was used to perform the mouthwash. During the second consecutive day, all tests were repeated in the same fashion (i.e. two multiple-flow measurements of  $F_{\text{ENO}}$  with different mouthwashes, totalling two arrays of testing on each day). The mouthwashes were not randomized, since the carbonated water mouthwash has a significantly longer effect on  $F_{\text{ENO}}$  than the tap water mouthwash [22].

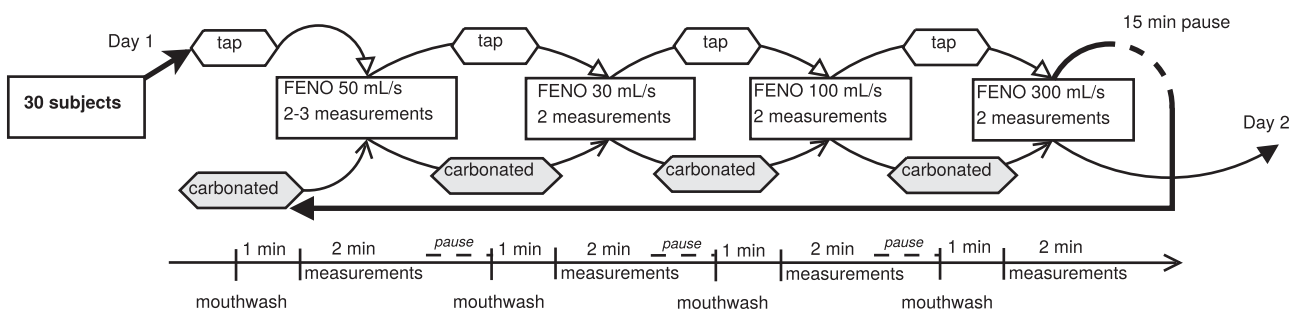
A minimum of two measurements of  $F_{\text{ENO}}$  at each flow were performed to obtain an acceptable value. The measurements at each stage were accepted if its variation was no more than 2 ppb. If more than two attempts were needed for getting a valid measurement, the oral rinsing was repeated. To further analyse the data, a mean value was obtained from four single determinations (two obtained each day) for every subject at each flow and mouthwash setting.

**Table 1.** Demographic, anthropometric and spirometric data of the study participants.

	<i>n</i>	Mean (SD)	Range	Spirometry	<i>n</i>	Mean (SD)	Range
Age (years)	30	43 (14)	16–68	FVC (l)	25	4.1 (1.1)	2.65–6.69
Height (cm)	30	171 (8)	157–186	FVC (% <sup>a</sup> )	25	93 (16)	50–120
Weight (kg)	30	73 (15)	49–109	FEV1 (l)	25	3.3 (0.9)	2.06–5.36
Female/male	17/13	57%/43%		FEV1 (% <sup>a</sup> )	25	93 (16)	50–117
Asthma	6	21%		FEV1/FVC ratio	25	0.80 (0.1)	0.69–0.98
Allergic rhinitis	9	31%		FEV1/FVC (% <sup>a</sup> )	25	98 (7)	88–125
Smoking	2	7%					
COPD	1	3%					

Data presented as mean (SD) or percentage of total case number.

<sup>a</sup>Percentage of predicted value (predicted according to Viljanen et al. [25]).



**Figure 1.** Flowchart illustrating the procedures, mouthwashes and repetitions performed during one day. Tap: tap water mouthwash, carbonated: carbonated water mouthwash.

The bottled carbonated water used for the mouthwash was commercially available (HARTWALL VICHY ORIGINAL<sup>®</sup>, Oy Hartwall Ab, Helsinki, Finland) and had a declared pH of 5.7–5.9; the tap water used had a pH of 8.3. More detailed information regarding the solutions can be found under Lassmann-Klee et al. [22].

All recommendations according to ATS/ERS were followed, except from the selection of a wide range of expiratory flows and using a mouthwash [5]. All subjects refrained from smoking four hours, from drinking coffee two hours and from eating and drinking one hour before the study. Also strenuous exercising prior measuring was discouraged.

Ethical committee approval was received (99/13/03/00/15) and we followed the ethical principles stated in the Declaration of Helsinki [26].

### Statistics

Analyses were performed using IBM<sup>®</sup> SPSS<sup>®</sup> statistics software version 22 (Armonk, NY), R<sup>®</sup> version 0.6.5 frontend and RSTUDIO<sup>®</sup> version 1.1.383 frontend to the R statistics language (THE R FOUNDATION<sup>®</sup>, Vienna, Austria), and we partially used GRAPHPAD<sup>®</sup> PRISM<sup>®</sup> version 5.04 to obtain the graphs (La Jolla, CA). The comparison of  $F_{\text{ENO}}$  was made using a Wilcoxon test for paired probes. We accepted a significance level of  $\alpha = 0.05$  as significant. The mean (SD) was obtained for each subject from four  $F_{\text{ENO}}$  measurements after each mouthwash at flows 30, 50, 100 and 300 mL/s, respectively. The median and the median absolute deviation (MAD) was calculated too [27]. To prove the accuracy of the measurements, we used the coefficient of variation ( $c_v$ ), defined as the quotient of standard deviation (SD) and mean. We defined a total mean value of  $c_v < 10\%$  as acceptable.  $C_{\text{ANO}}$ ,  $J'_{\text{awNO}}$  and  $D_{\text{awNO}}$  were calculated using a nonlinear logarithmic transformation (we used starting estimated values of the quadratic T transformation, a second order approximation) according to Eckel et al. [11] using following equation:

$$\log F_{\text{ENO}} = \log \left( \frac{J'_{\text{awNO}}}{D_{\text{awNO}}} + \left( C_{\text{ANO}} - \frac{J'_{\text{awNO}}}{D_{\text{awNO}}} \right)^{\frac{-D_{\text{awNO}}}{V}} \right) + \epsilon \quad (1)$$

$C_{\text{ANO}}$ ,  $J'_{\text{awNO}}$  or  $D_{\text{awNO}}$  were also calculated using the Högman and Meriläinen algorithm (HMA), with mean  $F_{\text{ENO}}$  and mean  $V'$  values for the flow rates: 30 mL/s, 100 mL/s

and 300 mL/s [12]. Cases with negative values for  $C_{\text{ANO}}$  and with failure of consistency check were not ruled out.

The comparison of  $C_{\text{ANO}}$ ,  $J'_{\text{awNO}}$  or  $D_{\text{awNO}}$  between mouthwashes was made using a Wilcoxon test for paired probes. All target variables were not normally distributed (Shapiro–Wilk's test). The differences between  $c_v$  were analysed with a Wilcoxon test when comparing mouthwashes. A comparison of all calculated  $c_v$  results was made with Friedman's two-way ANOVA.

### Results

The results of  $F_{\text{ENO}}$  measurements at multiple flow levels are listed in Table 2 and visualised in Figure 2. Individual  $F_{\text{ENO}}$  values are visualised in Figure 3.  $F_{\text{ENO}}$  ranged between 4.4 and 221.6 ppb and the median was 14.94 (MAD: 6.76) ppb at a flow rate of 50 mL/s after rinsing with tap water. The mouthwash with carbonated water reduced  $F_{\text{ENO}}$  significantly compared to the tap water mouthwash at every flow rate.

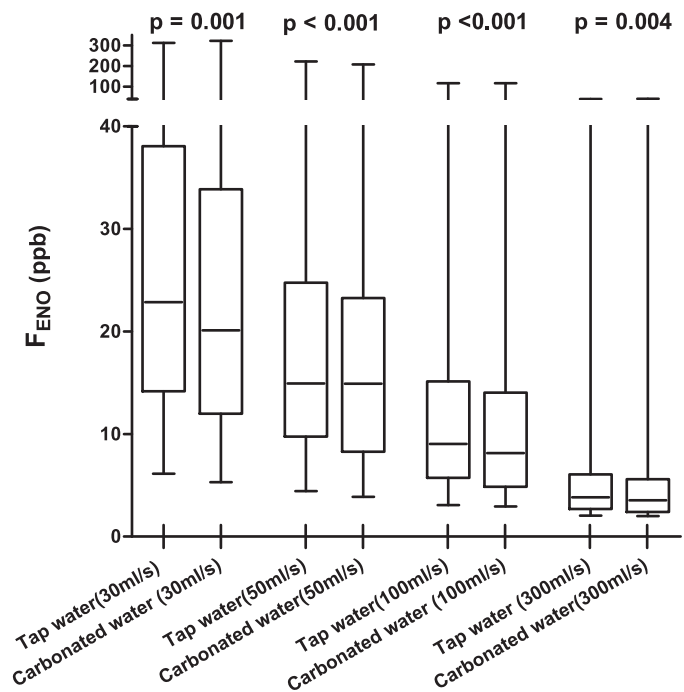


Figure 2.  $F_{\text{ENO}}$  measured at multiple expiratory flow levels after different mouthwashes. Pairs compared with the Wilcoxon signed-rank test.

Table 2. Summarized results of  $F_{\text{ENO}}$  at every flow rate and mouthwash either with tap water or carbonated water.

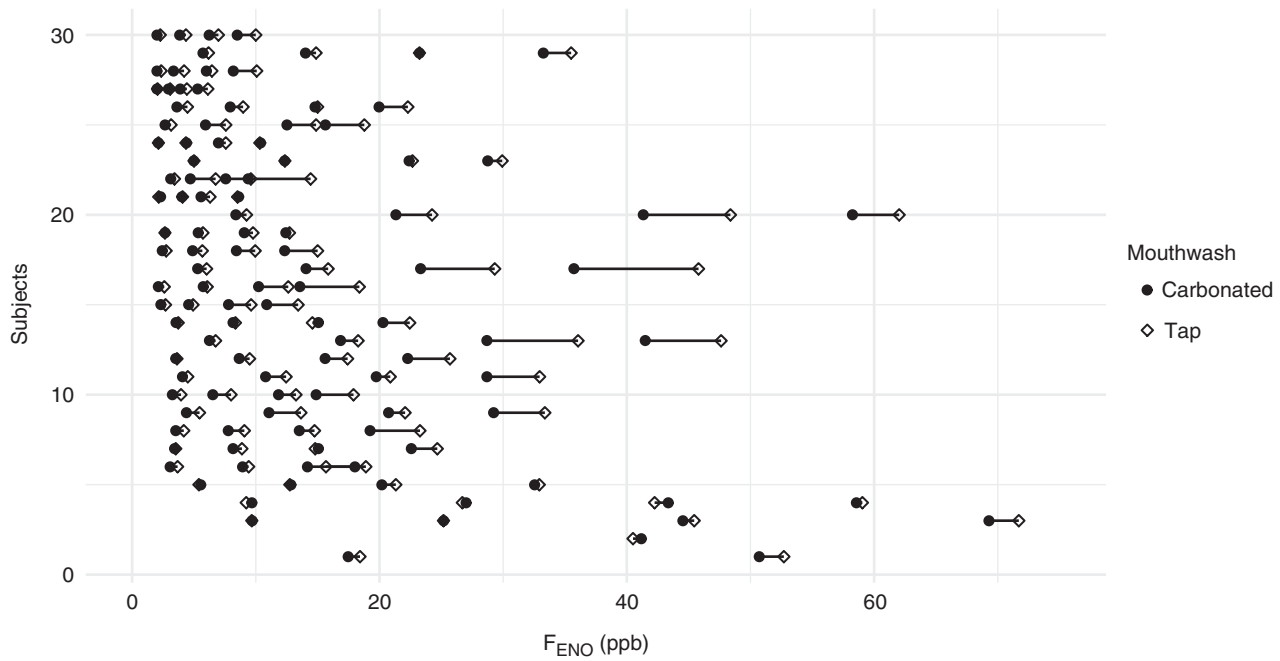
$F_{\text{ENO}}$ (ppb)	Median	MAD	IQR	$p^a$	Mean (SD)	Difference <sup>b</sup>	Mean $c_v^c$
Flow 30 mL/s, tap water	22.86	10.11	20.4	.001	39.21(57.04)	-4.6%	8.6 (4.5)
Flow 30 mL/s, carbonated water	20.11	9.49	20.7		37.40(59.28)		7.3 (5.0)
Flow 50 mL/s, tap water	14.94	6.76	13.3	<.001	27.27(40.19)	-6.4%	8.4 (6.2)
Flow 50 mL/s, carbonated water	14.91	7.24	14.4		25.51(38.01)		6.6 (3.9)
Flow 100 mL/s, tap water	9.03	3.91	8.8	<.001	15.41(21.58)	-4.4%	7.0 (6.6)
Flow 100 mL/s, carbonated water	8.15	3.68	8.7		14.74(21.72)		6.1 (4.0)
Flow 300 mL/s, tap water	3.84	1.36	3.2	.004	6.03(7.29)	-4.2%	6.9 (4.1)
Flow 300 mL/s, carbonated water	3.53	1.31	3.0		5.77(7.45)		8.0 (5.3)

Data presented as median, median absolute deviation (MAD), interquartile range (IQR),  $n = 30$ . Mean  $c_v$  calculated from individual  $c_v$  of  $F_{\text{ENO}}$  (consisting of at least four valid  $F_{\text{ENO}}$  results for each flow rate and mouthwash).

<sup>a</sup>Wilcoxon's signed ranks test.

<sup>b</sup>Relative decrease of the mean.

<sup>c</sup>Presented as mean (SD) in percent.



**Figure 3.** Individual  $F_{\text{ENO}}$  values measured at multiple expiratory flow levels after different mouthwashes. Ordinate represents the individuals ( $n = 30$ ), abscissa represents  $F_{\text{ENO}}$  (ppb) at multiple expiratory flow levels after: carbonated water mouthwash (black dots), and tap water mouthwash (grey rhombi). Abscissa truncated at 75 ppb. Subjects 1 and 2 had higher  $F_{\text{ENO}}$  (ppb) values (not shown). The missing higher values (tap water/carbonated) for subject 1 are: 128/128, 85/79; and for subject 2 are: 311/322, 222/208, 117/117.

**Table 3.** Results of  $J'_{\text{awNO}}$ ,  $C_{\text{ANO}}$  and  $D_{\text{awNO}}$ , after carbonated water or tap water mouthwash.

	$J'_{\text{awNO}}$ (pL/s)	$p^a$	$C_{\text{ANO}}$ (ppb)	$p^a$	$D_{\text{awNO}}$ (pL·s <sup>-1</sup> ·ppb <sup>-1</sup> )	$p^a$
Tap water	836.36 (457.58)	.015	0.88 (0.48)	.715	14.19 (7.52)	.299
Carbonated water	829.97 (443.12)		0.92 (0.33)		18.60 (3.87)	
Tap water <sup>b</sup>	796.88 (449.86)	<.001	1.00 (0.39)	.598	15.63 (6.95)	.871
Carbonated water <sup>b</sup>	724.96 (392.60)		1.04 (0.30)		15.28 (3.67)	

Data presented as median (median absolute deviation),  $n = 30$ .

<sup>a</sup>Wilcoxon's signed ranks test.

<sup>b</sup>Calculated with Högman and Meriläinen algorithm.

The mean  $c_v$  of  $F_{\text{ENO}}$  stayed under 10% for all flow levels. Comparing the  $c_v$  between tap water rinsing and carbonated water rinsing, no significant difference was found at any flow level (at 30 mL/s  $p = .094$ ; at 50 mL/s  $p = .125$ ; at 100 mL/s  $p = .245$ ; at 300 mL/s  $p = .688$ ). Analysing  $c_v$  for all flow levels and mouthwashes resulted in no difference ( $p = .202$ ).

When comparing  $J'_{\text{awNO}}$  between mouthwashes, the median  $J'_{\text{awNO}}$  for carbonated water was significantly lower using both models.  $C_{\text{ANO}}$  and  $D_{\text{awNO}}$  did not differ significantly between both mouthwashes. These results are summarized in Table 3.

The NO fraction of inspired gas was at all times below 20 ppb as recommended, having a mean value of 0.35 ppb (SD 0.34) [5].

## Discussion

### Mouthwashes

We found a statistically significant difference in  $F_{\text{ENO}}$  between mouthwashes at all expiratory flow levels.  $F_{\text{ENO}}$  was statistically significantly lower after rinsing with carbonated water compared to tap water at all expiratory flow levels.

This confirms previous studies in which carbonated water was used to perform a mouthwash [22,23], and suggests endorsement of a carbonated water mouthwash prior multiple-flow testing.

In the present study, the difference between mouthwashes was ca. -5% at all flow levels. This decrease of  $F_{\text{ENO}}$  between mouthwashes equals our previously observed reduction. We demonstrated recently that both carbonated water and tap water mouthwashes lower  $F_{\text{ENO}}$  significantly compared with the baseline and observed an immediate decrease of  $F_{\text{ENO}}$  after a carbonated water mouthwash of -18% and of -13% after a tap water mouthwash [22]. Additionally, Piirilä et al. [23] found at 50 mL/s a decrease from baseline (without mouthwash) of ca. -10% after a carbonated water mouthwash in a healthy population. In comparison, previous multiple flow studies have found a relative decrease in  $F_{\text{ENO}}$  of ca. -10% after a chlorhexidine mouthwash in children and adolescent (both asthmatic and healthy) [28] and of ca. -15% in healthy adults [29].

Furthermore, the tap water mouthwash's effect is short-lasting, only two minutes. On the other hand, the carbonated water mouthwash's effect is longer, lasting 12 minutes, and more effective if compared with tap water [22]. For this

reason, we did not randomize the subjects in the present study and performed the measurements with tap water first.

Our data were accurately collected, since the intra-individual  $c_v$  of  $F_{\text{ENO}}$  stayed low and the mean  $c_v$  below 10%. We did not find a difference in  $c_v$  between mouthwashes. The  $c_v$  at 100 mL/s is similar to previously reported by Ekroos et al. [30] for a healthy male population taking into account a time period of maximal 24 hours.

### Multiple flow $F_{\text{ENO}}$ and mouthwashes

As we expected,  $J'_{\text{awNO}}$  was significantly lower after the carbonated water mouthwash compared to the tap water mouthwash. There was no statistically significant difference between mouthwashes when analysing  $C_{\text{ANO}}$  and  $D_{\text{awNO}}$ . We verified this results with estimated values for  $J'_{\text{awNO}}$ ,  $C_{\text{ANO}}$  and  $D_{\text{awNO}}$  and two different models. This further strengthens our result, that the carbonated water mouthwash affects the airway fraction (due to oral NO reduction) and not the peripheral alveolar NO, neither the NO diffusion. We could state the hypothesis that the carbonated water rinsing provides more exact values (without oral contamination) in general. Heijkenskjöld-Rentzhog et al. published similar findings when analysing  $J'_{\text{awNO}}$  and  $C_{\text{ANO}}$  after a chlorhexidine mouthwash [28]. This is in contrast to results by Malinovschi et al. [29] who found also a decrease in  $C_{\text{ANO}}$  after a chlorhexidine mouthwash. Both used a trumpet-shaped model with corrections for axial diffusion (TMAD). Kerckx et al. [31] demonstrated that, when using a model correcting for axial diffusion to estimate  $C_{\text{ANO}}$  in asthmatic patients (unobstructed and well-managed),  $C_{\text{ANO}}$  is normal, even when  $J'_{\text{awNO}}$  is elevated. This has been confirmed also for patients with severe asthma and during exacerbation [32].

For our purposes, we employed a two compartment model of the lung [4], without considering axial diffusion [9], and applied a robust mathematical model [11]. This model was tested previously exactly at the same flow levels by Eckel et al. and did not impose flow limitations unlike other models [11]. We obtained also values using the HMA (without axial diffusion) and the results between mouthwashes were similar. Although results from models with and without axial diffusion are not directly equivalent, an analogy can be made between the main findings of different studies.

The strength of our study was using different parameters to examine the effect of the carbonated water mouthwash and for that using two different mathematical models. Our calculations with Equation (1) provided negative values for  $C_{\text{ANO}}$  in five cases for tap water mouthwash and four cases for carbonated water mouthwash, which were not excluded. A similar amount of negative values were obtained with the HMA. This has been a regular problem with other models providing a greater proportion of negative concentration values. We disregarded the importance of these data, since the comparison of  $C_{\text{ANO}}$  between mouthwashes showed no difference (even when replacing these values for zero). Our extended analysis of  $J'_{\text{awNO}}$ ,  $C_{\text{ANO}}$  and  $D_{\text{awNO}}$  found these

values comparable to previous results in a healthy population [33]. All results for  $C_{\text{ANO}}$  were under 2.3 ppb and none of the subjects had a medical condition associated with interstitial lung disease, therefore we assume that the subjects with measured  $F_{\text{ENO}}$  over 25 ppb ( $n=7$ ) had an elevated airway production of NO.

### Limitations and implications

We acknowledge the limitation of not randomizing the order of the expiratory flows levels. However, the limitation was imposed by the nature of the flow levels used, i.e. the high flow representing an expiratory burden to the participants. Previous studies reported a decrease in  $F_{\text{ENO}}$  after forced expirations [34–36]. We selected on purpose an incremental flow order to avoid the high flow expiration influencing the slower expiration manoeuvres.

The tap water used to compare the effect of carbonated water had a pH value over 8. The tap water in European countries has a pH of 6.5–9.5 units (Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption) and this range also complies with WHO guidelines. Many countries may have lower tap water's pH values than 8. This may enhance the tap water's effect on oral  $F_{\text{ENO}}$  and lower the differences between tap water and carbonated water. Nevertheless, further studies are needed to elucidate if the pH is the crucial factor of the mouthwash solutions.

Carbonated water poses an ideal candidate for performing a mouthwash before multiple flow measuring. It eliminates oral NO interference more effectively than tap water and for a prolonged period of time. Our recent publication [22] argues for the clinical application of a mouthwash before  $F_{\text{ENO}}$  measurement. Here, we demonstrate it influences only the airway fraction of  $F_{\text{ENO}}$ , and using carbonated water as a mouthwash, in a more pronounced way. Probably one mouthwash procedure with carbonated water may suffice when performing a routine multiple-flow investigation, but repetitions might be useful if exact values are needed, e.g. in physiological research.

### Conclusions

We conclude that a carbonated water mouthwash can significantly reduce oropharyngeal NO compared to a tap water mouthwash at expiratory flows of 30–300 mL/s without affecting the  $C_{\text{ANO}}$  nor the  $D_{\text{awNO}}$ . We imply that a carbonated water mouthwash is suitable for routine multiple-flow  $F_{\text{ENO}}$ -analysis and evidently useful in clinical research. This study strengthens the view that mouthwash procedures shall be taken into account when comparing  $F_{\text{ENO}}$  values.

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## Disclosure statement

No conflicts of interest are declared by the author(s).

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