

## Original Article



# Influence of oral care on fractional exhaled nitric oxide

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## ABSTRACT

**Background:** Fractional exhaled nitric oxide (FeNO) is an indicator of bronchial inflammation in asthma patients. However, nitric oxide is also produced in the oral cavity, with production depending on the local anaerobic flora and intraoral acidity.

**Objective:** To evaluate the influence of oral care on measurement of FeNO, to investigate the influence of sleep when the oral environment changes dramatically, and to assess the impact of oral care on FeNO in the real clinical setting.

**Methods:** FeNO was measured before and after oral care in 14 subjects on awakening and at bedtime on 2 consecutive days to investigate variation of nitric oxide derived from the oral cavity. It was also measured before and after oral care in 62 outpatients with asthma to assess the clinical relevance of oral cavity nitric oxide.

**Results:** On both days, FeNO was significantly decreased by oral care on awakening (day 1: decrease =  $10.6 \pm 12.4$  ppb,  $p = 0.0020$ ; day 2: decrease =  $11.6 \pm 23.7$  ppb,  $p = 0.0009$ ), and the decrease was larger than at bedtime. In addition, FeNO was significantly reduced by oral care in asthma outpatients (decrease =  $1.73 \pm 0.95$  ppb,  $p = 0.0090$ ), and older age was significantly correlated with the decrease ( $p = 0.0261$ ).

**Conclusion:** Oral care resulted in a decrease of FeNO, especially on awakening. While nitric oxide derived from the oral cavity generally has a limited impact in outpatients with asthma, its influence on measurement of FeNO may need to be considered, especially in elderly patients.

**Keywords:** Asthma; Bacteria; Nitric oxide; Oral hygiene

## INTRODUCTION

Bronchial asthma is an inflammatory disease of the airways and fractional exhaled nitric oxide (FeNO) is widely used as a noninvasive indicator of airway inflammation [1, 2]. While FeNO does not purely reflect lower airway production of nitric oxide (NO) since it is also produced in the oral cavity, a study has shown that less than 7.5% of total FeNO originates from the oral cavity [3]. The oral anaerobic bacterial flora and acidity are known to be key factors that influence NO production in the oral cavity. Nitrate is abundant in green vegetables and is selectively concentrated in the salivary glands after being absorbed from the digestive tract [4-6]. Nitrate secreted in the saliva undergoes reduction to nitrite by anaerobic bacteria in

the oral cavity, and then is reduced further to NO by oral acidity. These are nonenzymatic reactions, and changes of various oral factors can alter local NO production [4-8].

Bacteria are abundant in the upper airway [9], with the bacterial flora being increased by poor oral hygiene [9, 10] and decreased by good oral care [11, 12]. In particular, bacteria grow rapidly in biofilms that form on dental plaque [9] and oral cavity NO production is increased in the presence of plaque [7, 8]. The condition of the oral cavity changes throughout the day. During sleep, production of saliva is reduced and clearance of the oral cavity is suppressed, while bacterial growth is promoted in a more acidic intraoral environment [12-14]. These factors that promote oral NO production might have the maximum impact at the time of awakening. Accordingly, we predicted that FeNO may be higher at awakening than during the daytime when oral cavity clearance is improved by daily activities such as eating or brushing the teeth.

In the present study, we evaluated the influence of oral care on FeNO in healthy volunteers and asthma patients immediately after awakening and at bedtime, as well as in asthma patients attending our outpatient department during the daytime.

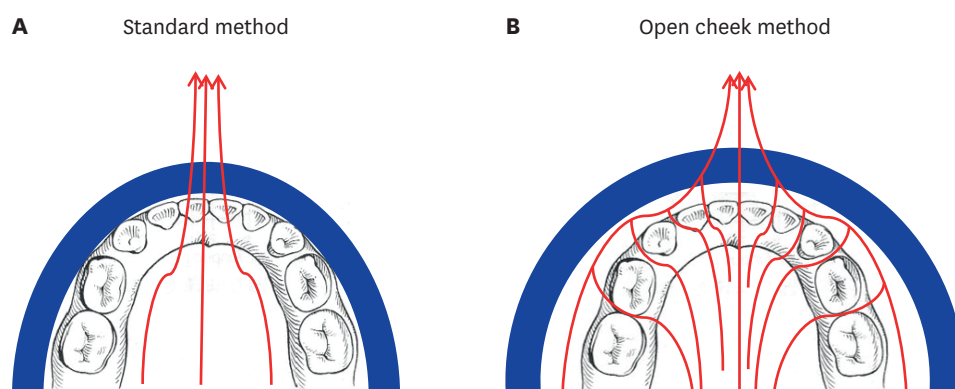
## MATERIALS AND METHODS

### Study 1

FeNO was measured on awakening and at bedtime on 2 consecutive days to investigate its variation. From March to September 2010, staff of the National Hospital Organization Disaster Medical Center and their family members were recruited, including 11 healthy subjects and 3 subjects with a history of asthma. In each subject, measurement of FeNO was performed 3 times (before oral care, after gargling with water, and after brushing the teeth). As factors that might influence oral production of NO, intake of green vegetables on the previous day (yes/no) and the existence of periodontitis (yes/no) were assessed by inquiry. The Niox Mino device (Aerocrine AB, Stockholm, Sweden) was used for measurement of FeNO at home by each subject.

### Study 2

This study was performed to investigate the impact of oral care on FeNO in the real clinical setting. From January to April 2012, asthma patients who were attending the outpatient clinic of the National Hospital Organization Disaster Medical Center were recruited. At an outpatient visit between 9 AM and 4 PM, FeNO was measured before and after oral care (brushing the teeth for 3 minutes, followed by gargling with water). To evaluate the influence on FeNO of increased exposure of exhaled air to the teeth and gingivae, measurement was done in 2 ways (**Fig. 1**): (A) the standard method with no gap between the teeth and buccal mucosa, and (B) the open cheek method with a gap between the teeth and buccal mucosa and the teeth clenched to maximise exposure of exhaled air to the teeth and the gingivae. Patients were asked about the intake of green vegetables on the previous day (yes/no), the existence of periodontitis (yes/no), and the time since last brushing the teeth, the time since the last meal, and the time since last brushing the teeth or eating (as factors that might influence oral cavity hygiene). The following parameters were also investigated on the day of FeNO measurement: total IgE (IU/mL), peripheral blood eosinophil count (%), sputum eosinophils (positive/negative), forced expiratory volume (FEV)<sub>1.0</sub> (mL), and the Asthma Control Test (ACT) score.



**Fig. 1.** Measurement of fractional exhaled nitric oxide was done by 2 methods. (A) Standard method with no gap between the teeth and buccal membrane. (B) Open cheek method with a gap between the teeth and buccal membrane and the teeth clenched to maximise exposure of exhaled air to the surface of the teeth and the gingivae.

### Statistics

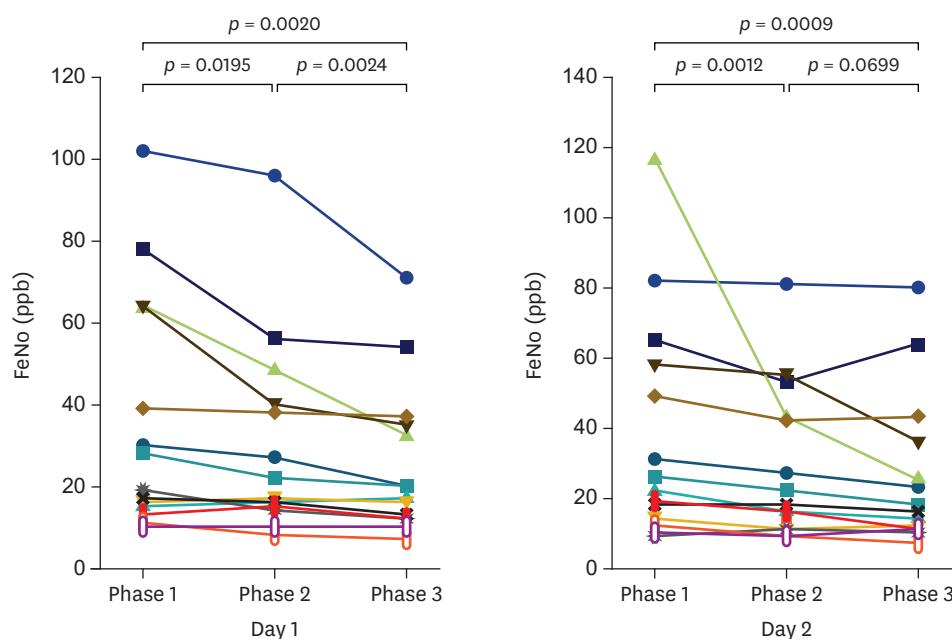
FeNO levels before and after oral care were analyzed by the Wilcoxon signed rank test in studies 1 and 2. In study 2, the relationship between the FeNO level and various factors was assessed by using the Wilcoxon rank sum test for sputum eosinophils and by Spearman rank correlation coefficient analysis for peripheral blood eosinophils, IgE, FEV1.0%, ACT score, and age. The relationship between the change of FeNO after oral care and various factors was analyzed by the Wilcoxon rank sum test for green vegetable intake and periodontitis, while Spearman rank correlation coefficient analysis was employed for the age, time after last brushing the teeth, time after the last meal, and time since last brushing the teeth or eating. Furthermore, logistic regression analysis (multivariate analysis) was done to investigate all of these factors. Calculations were performed by using JMP for Windows version 10 (SAS Institute Japan, Tokyo, Japan). All  $p$  values were 2-tailed and  $p < 0.05$  was considered to indicate statistical significance.

This study was approved by the ethics committee of the National Hospital Organization Disaster Medical Center and written informed consent was obtained from all participants. This study has been registered with the University Hospital Medical Information Network (UMIN) clinical trials registry (registration numbers are UMIN000017046 for study 1 and UMIN000017427 for study 2).

## RESULTS

### Study 1

The median age of the 14 subjects (3 women and 11 men) was 34 years (range, 25–83 years). On day 1, one subject failed to measure FeNO at night, one failed to measure it after gargling in the morning, and 2 failed to do so after gargling at night. In one subject, the FeNO titer after gargling at night was  $<5$  ppb (below the lower limit of measurement), and this result was excluded from statistical analysis since the exact titer was unknown. Accordingly, complete day 1 data after gargling were available for 13 subjects in the morning and 10 subjects at night, while complete data after oral care were available for 14 subjects in the morning and 13 subjects at night. On day 2, one subject failed to measure FeNO after gargling at night.



**Fig. 2.** Influence of oral care on the morning FeNO level. On both days, the FeNO level measured on awakening was significantly decreased by oral care (gargling plus brushing the teeth) and by gargling alone. After gargling, FeNO showed a further significant decrease with brushing the teeth on day 1, but not on day 2 (Wilcoxon signed rank test). FeNO, fractional exhaled nitric oxide. Phase 1: before oral care. Phase 2: after gargling with water. Phase 3: after brushing the teeth.

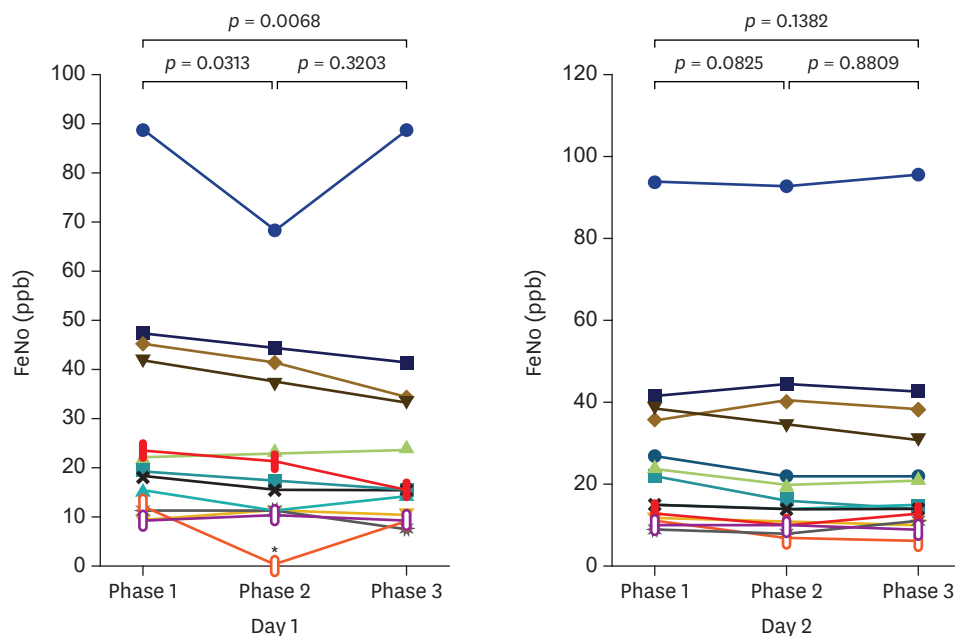
**Fig. 2** shows the change of FeNO after oral care in the morning on days 1 and 2. On both days, FeNO was significantly decreased by oral care gargling plus brushing the teeth (day 1:  $n = 14$ ,  $\Delta\text{FeNO} = 10.6 \pm 12.4$  ppb,  $p = 0.0020$ ; day 2:  $n = 14$ :  $\Delta\text{FeNO} = 11.6 \pm 23.7$  ppb,  $p = 0.0009$ ) and by gargling alone before brushing the teeth (day 1:  $n = 13$ ,  $\Delta\text{FeNO} = 5.2 \pm 8.4$  ppb,  $p = 0.0195$ ; day 2:  $n = 14$ ,  $\Delta\text{FeNO} = 8.5 \pm 19.2$  ppb,  $p = 0.0012$ ). After gargling, FeNO showed a further significant decrease with brushing the teeth on day 1 ( $n = 13$ ,  $\Delta\text{FeNO} = 3.92 \pm 6.66$  ppb,  $p = 0.0024$ ), but not on day 2 ( $n = 14$ ,  $\Delta\text{FeNO} = 3.1 \pm 7.6$  ppb,  $p = 0.0699$ ). **Fig. 3** shows the change of FeNO due to oral care before bedtime on days 1 and 2. On day 1, FeNO decreased significantly after oral care ( $n = 13$ ,  $\Delta\text{FeNO} = 3.6 \pm 3.9$  ppb,  $p = 0.0068$ ), as well as after gargling alone ( $n = 10$ ,  $\Delta\text{FeNO} = 3.5 \pm 6.2$  ppb,  $p = 0.0313$ ). In contrast, oral care did not significantly influence the FeNO level on day 2 ( $n = 14$ ,  $\Delta\text{FeNO} = 1.7 \pm 3.5$  ppb,  $p = 0.1382$ ).

When the variation of FeNO values measured in the morning and at night on days 1 and 2 was assessed in each subject, the absolute value of (FeNO on day 1 – FeNO on day 2) was significantly decreased by oral care in the morning ( $n = 14$ ,  $p = 0.0234$ ), but not at night ( $n = 13$ ,  $p = 0.4727$ ) (**Fig. 4**).

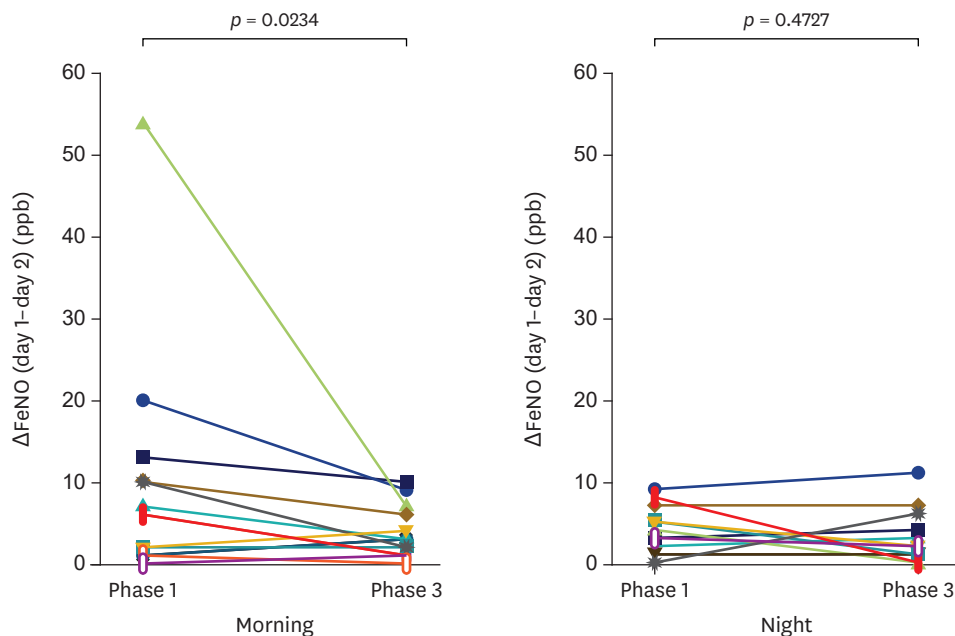
Among the 14 subjects, 3 had periodontitis and 5 had eaten green vegetables on the previous day. However, the decrease of FeNO was not correlated with either periodontitis or green vegetable intake, as well as not being correlated with age.

## Study 2

Sixty-two asthma patients were recruited for this study, including 22 men and 40 women aged 24–82 years (median age, 61.5 years) (**Table 1**).



**Fig. 3.** Influence of oral care on the nighttime FeNO level. On day 1, the FeNO level measured at bedtime showed a significant decrease after oral care (gargling plus brushing the teeth) and after gargling alone. On day 2, oral care did not have a significant influence on FeNO (Wilcoxon signed rank test). FeNO, fractional exhaled nitric oxide. Phase 1: before oral care. Phase 2: after gargling with water. Phase 3: after brushing the teeth. \*The FeNO titer was <5 ppb (below the lower limit of measurement), and this result was excluded from statistical analysis.



**Fig. 4.** Intrasubject variation of morning and nighttime FeNO levels between day 1 and day 2. On awakening, the absolute value of (FeNO on day 1 - FeNO on day 2) was significantly decreased by oral care, but not at bedtime (Wilcoxon signed rank test). FeNO, fractional exhaled nitric oxide. Phase 1: before oral care. Phase 3: after oral care. The vertical axis displays absolute value of (FeNO on day 1 - FeNO on day 2).

**Table 1.** Patients' characteristics

Characteristic	Value
Age (yr)	61.5 (24–82)
Sex (n)	
Male	40
Female	22
Smoking status (n)	
Never	40
Ex-smoker	20
Current	2
Allergic predisposition (n)	
Allergic rhinitis	26
Atopic dermatitis	2
IgE (IU/mL)	170.5 (1–25,327)
ICS dose (n)	
Naïve	15
Low	9
Moderate	19
High	19
Oral steroid (n)	3
GINA steps (n)	
1	4
2	13
3	21
4	22
5	2

Values are presented as median (range) unless otherwise indicated.

GINA, Global Initiative for Asthma.

#### *FeNO before and after oral care*

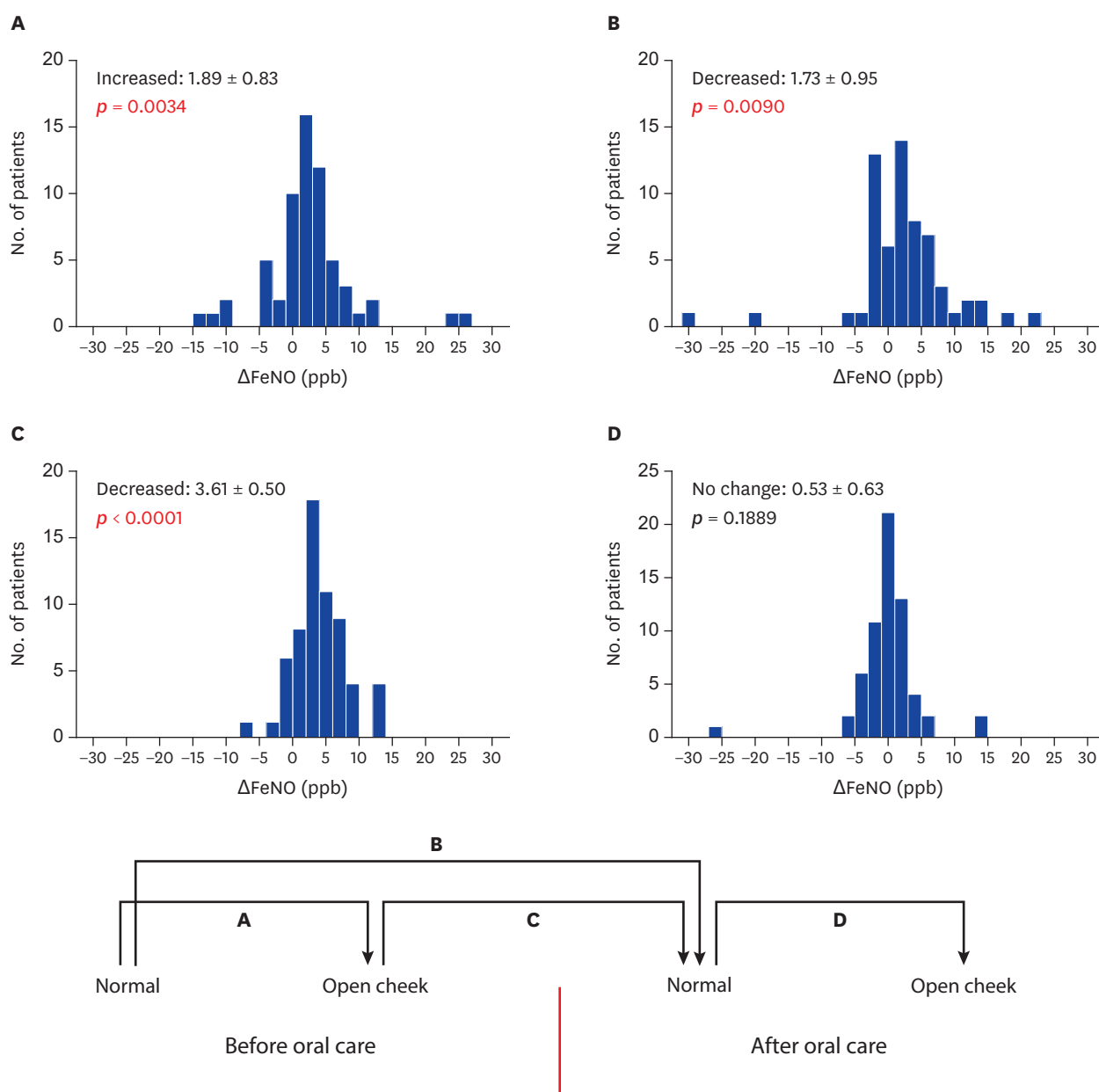
**Fig. 5** shows the changes of FeNO with oral care in the 62 asthma patients. Before oral care, FeNO was significantly higher when measured by the open cheek method compared with the standard method ( $\Delta\text{FeNO} = 1.89 \pm 0.83$  ppb,  $p = 0.0034$ ), but the difference between these 2 methods was not significant after oral care ( $\Delta\text{FeNO} = 0.53 \pm 0.63$  ppb,  $p = 0.1889$ ). FeNO measured by the standard method was significantly reduced by oral care ( $\Delta\text{FeNO} = 1.73 \pm 0.95$  ppb,  $p = 0.0090$ ). The percent decrease of NO was  $3.4\% \pm 26.8\%$  and it varied widely between the subjects. The greatest decrease was observed between FeNO measured by the open cheek method before oral care and FeNO measured by the standard method after oral care ( $\Delta\text{FeNO} = 3.61 \pm 0.50$  ppb,  $p < 0.0001$ ). Thus, greater contact of expired air with the teeth and gingivae increased FeNO before oral care, while oral care reduced FeNO and there was no significant difference between the 2 methods of measurement after oral care.

#### *FeNO measured by the standard method and asthma parameters*

**Table 2** shows the relationship between FeNO measured by the standard method and age or parameters of asthma activity. The peripheral eosinophil count (%) and IgE were significantly correlated with FeNO before and after oral care.

#### *Factors increasing NO derived from the oral cavity*

**Table 3** shows the relationship between reduction of FeNO measured by the standard method after oral care ( $\Delta\text{FeNO}_{\text{after-before}}$ ), which might reflect the proportion of total NO derived from the oral cavity, and factors that might influence oral production of NO, including green vegetable intake on the previous day, periodontitis, age, time since last brushing the teeth, time since the last meal, and time since last brushing the teeth or eating. Among these



**Fig. 5.** (A) Influence of oral care on FeNO measured by 2 different methods (Wilcoxon signed rank test). Greater contact of expired air with the teeth and gingivae led to an increase of FeNO before oral care. (B) Oral care decreased FeNO. (C) The maximum decrease was noted when the open cheek method before oral care was compared with the standard method after oral care. (D) After oral care, there was no significant difference of FeNO between the 2 methods of measurement. FeNO, fractional exhaled nitric oxide.

**Table 2.** FeNO measured by the standard method and asthma parameters

Variable	FeNO level (ppb)	
	Before oral care ( $p$ value)	After oral care ( $p$ value)
Eosinophils in the sputum (+/-) ( $n = 54$ )	0.2977	0.3613
Peripheral blood eosinophil count (%) ( $n = 62$ )	0.0003	<0.0001
IgE (IU/mL) ( $n = 62$ )	0.0018	0.0058
FEV1.0% ( $n = 57$ )	0.0982	0.2509
ACT score ( $n = 62$ )	0.3869	0.1139
Age ( $n = 62$ )	0.6229	0.8509

FeNO, fractional exhaled nitric oxide; FEV, forced expiratory volume; ACT, Asthma Control Test.



**Table 3.** Factors potentially related to the decrease of fractional exhaled nitric oxide after oral care

Variable	<i>p</i> value	
	Univariate analysis	Multivariate analysis
Periodontitis (+/-) (n = 62)	0.3154	0.0773
Green vegetable intake (n = 62)	0.9598	0.6833
Age (n = 62)	0.0365	0.0261
Time after last brushing the teeth (hr) (n = 62)	0.6619	0.7650
Time after the last meal (hr) (n = 60)	0.7612	0.3552
Time after last brushing the teeth or eating (hr) (n = 60)	0.8600	0.6176

factors, older age was significantly correlated with  $\Delta\text{FeNO}_{\text{after-before}}$  according to univariate analysis ( $p = 0.0365$ ) and also by multivariate analysis ( $p = 0.0261$ ).

## DISCUSSION

Our findings suggested that oral care might influence FeNO by reducing the amount of NO originating from the oral cavity, possibly by removal of bacteria and dental plaque along with reduction of intraoral acidity. The percentage of NO derived from the oral cavity was larger on awakening in the morning than during the daytime or at night, which could be explained by the maximum bacterial load and maximum acidity being reached during sleep, although these factors were not examined directly in our study. Carossa reported production of NO by bacteria in oral plaque and found that FeNO was  $58.8 \pm 3.7$  ppb in healthy young adults who performed no oral care for 24 hours, while it decreased to  $43.6 \pm 3.7$  ppb after brushing the teeth [8]. The marked reduction of FeNO after oral care observed by Carossa was consistent with our results obtained immediately after awakening in study 1. Compared with immediately after awakening, production of NO may decrease in the oral cavity during the daytime due to daily activities such as brushing the teeth or intake of food and beverages, as well as increased production of saliva. The percentage of NO derived from the oral cavity showed wide variation among our subjects (Fig. 2), and some of them were high oral cavity FeNO producers. Also, intraindividual variation of oral cavity FeNO production was highest in the morning and was reduced after oral care, but variation was not reduced by oral care at nighttime (Fig. 4). These results suggest that the bacterial load and oral cavity acidity with resultant FeNO production can vary from day to day or even from hour to hour. In study 2, the mean reduction of FeNO by oral care was statistically significant, but was generally not clinically relevant. However, the reduction of FeNO by oral care was  $>10$  ppb in 7 patients (11.3%), which was large enough to potentially influence clinical judgment.

The method of measuring FeNO might also influence the data obtained, as shown in study 2. However, FeNO levels measured after oral care were similar on 2 consecutive days in study 1, and also did not show a significant difference between the 2 methods of exhalation in study 2. Thus, the FeNO level measured after oral care seems to be relatively reproducible and might reflect NO production in the lower airways more closely than that measured before oral care.

Whether FeNO is influenced by age remains controversial [15], with no relationship to age being found in healthy adults by one study [16], while other studies have shown that FeNO increases with age (especially over 60 years old) in a general population including subjects with asthma [17] or in healthy subjects [18]. The mechanism underlying the age-dependent increase of FeNO is unknown, but it has been suggested to involve elevation of the inflammatory response or reduced lung diffusing capacity for nitric oxide in the elderly [17, 18]. In study 2,



there was no relationship between the FeNO level and age in asthma patients both before and after oral care, but we found that a higher proportion of NO was derived from the oral cavity in older subjects. The number and abundance of bacterial species both increase with aging and secretion of saliva is reduced in the elderly [19, 20], promoting an acidic intraoral environment [12, 20]. Since a heavier bacterial load and increased acidity are key factors related to NO production in the oral cavity, it would seem reasonable for more NO to originate from the oral cavity in elderly persons than in younger persons. Thus, care should be taken when measuring FeNO in elderly patients, since misdiagnosis between asthma and chronic obstructive pulmonary disease could potentially occur if oral NO production is ignored.

Based on the results of study 1, brushing the teeth should be performed for oral care rather than just gargling to allow precise evaluation of lower respiratory tract inflammation by measurement of FeNO, possibly because brushing reduces dental plaque. Thus, a standard oral care regimen should be established to minimise NO derived from the oral cavity in patients undergoing FeNO measurement.

This study had some limitations. We found that intake of green vegetables was not significantly correlated with the amount of NO derived from the oral cavity, and detection of eosinophils in sputum was also not correlated with the FeNO level. These relationships might not have been identified because we could not quantify green vegetable intake or the sputum eosinophil count. Green vegetables have a high content of nitrate, which is eventually secreted into the saliva and is subsequently reduced to NO in the oral cavity [4-6]. However, dietary NO only shows an influence within 4 hours of intake [8] and might have little effect on awakening in the morning. A second limitation of this study was self-reporting of periodontitis, which is associated with a high oral bacterial load, rather than diagnosis of this condition by dentists. Third, the subjects measured FeNO at home without supervision in study 1 and there were some missing data.

Reproducibility of FeNO measurement with the Niox Mino device used in this study is reported to be high [21, 22], with a change of the FeNO value by  $\geq 4$  ppb indicating a change of inflammatory status [21]. However, some of our results might represent outliers. For example, one subject showed reduction of FeNO by 10 ppb (-15.4%) after gargling in the morning on day 2, and one subject showed reduction by 20 ppb (-22.7%) after gargling at night on day 1, despite the FeNO titer after brushing the teeth being almost the same as before oral care in both cases. These results cannot be explained by improvement of the oral environment or a change of inflammatory status and might be due to the influence of other unknown factors. Study 2 included 2 current smokers. FeNO may be falsely low in current smokers due to down-regulation of NOS and enhanced local clearance of NO in the lower airway [15]. However, this study was performed to evaluate NO production in the oral cavity rather than the lungs, so participation of current smokers should not have influenced the results. In both studies 1 and 2, oral care by the subjects was not regulated. Daytime oral care could have influenced the nighttime oral NO level in study 1 and morning oral care could have affected the results of study 2. However, univariate and multivariate analysis both indicated that the time since last brushing the teeth did not affect oral NO production, suggesting that lack of brushing in the morning might be compensated by oral clearance due to food intake and enhanced salivation.

In conclusion, oral care decreased FeNO in both healthy subjects and asthma patients. The proportion of NO derived from the oral cavity was highest immediately after awakening, and

was smaller when FeNO was measured at the outpatient clinic in the daytime. However, some subjects still showed high oral NO production in the daytime, particularly older patients. Thus, NO production in the oral cavity should be taken into consideration when FeNO is unexpectedly high relative to a patient's symptoms and clinical status. To avoid the possible influence of NO derived from the oral cavity, a standard regimen for performing oral care before measurement of FeNO should be established.

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## REFERENCES

1. Barnes PJ, Dweik RA, Gelb AF, Gibson PG, George SC, Grasemann H, Pavord ID, Ratjen F, Silkoff PE, Taylor DR, Zamel N. Exhaled nitric oxide in pulmonary diseases: a comprehensive review. *Chest* 2010;138:682-92.  
[PUBMED](#) | [CROSSREF](#)
2. Bjermer L, Alving K, Diamant Z, Magnussen H, Pavord I, Piacentini G, Price D, Roche N, Sastre J, Thomas M, Usmani O. Current evidence and future research needs for FeNO measurement in respiratory diseases. *Respir Med* 2014;108:830-41.  
[PUBMED](#) | [CROSSREF](#)
3. Silkoff PE, McClean PA, Caramori M, Slutsky AS, Zamel N. A significant proportion of exhaled nitric oxide arises in large airways in normal subjects. *Respir Physiol* 1998;113:33-8.  
[PUBMED](#) | [CROSSREF](#)
4. Zetterquist W, Pedroletti C, Lundberg JO, Alving K. Salivary contribution to exhaled nitric oxide. *Eur Respir J* 1999;13:327-33.  
[PUBMED](#) | [CROSSREF](#)
5. Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr* 2009;90:140.  
[PUBMED](#) | [CROSSREF](#)
6. Govoni M, Jansson EA, Weitzberg E, Lundberg JO. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric Oxide* 2008;19:333-7.  
[PUBMED](#) | [CROSSREF](#)
7. Schreiber F, Stief P, Gieseke A, Heisterkamp IM, Verstraete W, de Beer D, Stoodley P. Denitrification in human dental plaque. *BMC Biol* 2010;8:24.  
[PUBMED](#) | [CROSSREF](#)
8. Carossa S, Pera P, Doglio P, Lombardo S, Colagrande P, Brussino L, Rolla G, Bucca C. Oral nitric oxide during plaque deposition. *Eur J Clin Invest* 2001;31:876-9.  
[PUBMED](#) | [CROSSREF](#)
9. Mojon P. Oral health and respiratory infection. *J Can Dent Assoc* 2002;68:340-5.  
[PUBMED](#)
10. Gibbons RJ, Etherden I. Fibronectin-degrading enzymes in saliva and their relation to oral cleanliness. *J Periodontal Res* 1986;21:386-95.  
[PUBMED](#) | [CROSSREF](#)
11. Maeda K, Akagi J. Oral care may reduce pneumonia in the tube-fed elderly: a preliminary study. *Dysphagia* 2014;29:616-21.  
[PUBMED](#) | [CROSSREF](#)
12. Tada A, Miura H. Prevention of aspiration pneumonia (AP) with oral care. *Arch Gerontol Geriatr* 2012;55:16-21.  
[PUBMED](#) | [CROSSREF](#)

13. Bollen CM, Beikler T. Halitosis: the multidisciplinary approach. *Int J Oral Sci* 2012;4:55-63.  
[PUBMED](#) | [CROSSREF](#)
14. Hara AT, Zero DT. The potential of saliva in protecting against dental erosion. *Monogr Oral Sci* 2014;25:197-205.  
[PUBMED](#) | [CROSSREF](#)
15. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, Plummer AL, Taylor DR American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;184:602-15.  
[PUBMED](#) | [CROSSREF](#)
16. Tsang KW, Ip SK, Leung R, Tipoe GL, Chan SL, Shum IH, Ip MS, Yan C, Fung PC, Chan-Yeung M, Lam W. Exhaled nitric oxide: the effects of age, gender and body size. *Lung* 2001;179:83-91.  
[PUBMED](#) | [CROSSREF](#)
17. Olin AC, Rosengren A, Thelle DS, Lissner L, Bake B, Torén K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. *Chest* 2006;130:1319-25.  
[PUBMED](#) | [CROSSREF](#)
18. Gelb AF, George SC, Camacho F, Fraser C, Flynn Taylor C, Shakkottai S. Increased nitric oxide concentrations in the small airway of older normal subjects. *Chest* 2011;139:368-75.  
[PUBMED](#) | [CROSSREF](#)
19. Gonsalves WC, Wrightson AS, Henry RG. Common oral conditions in older persons. *Am Fam Physician* 2008;78:845-52.  
[PUBMED](#)
20. Gupta A, Epstein JB, Sroussi H. Hyposalivation in elderly patients. *J Can Dent Assoc* 2006;72:841-6.  
[PUBMED](#)
21. Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. *Eur Respir J* 2003;21:433-8.  
[PUBMED](#) | [CROSSREF](#)
22. Gill M, Graff GR, Adler AJ, Dweik RA. Validation study of fractional exhaled nitric oxide measurements using a handheld monitoring device. *J Asthma* 2006;43:731-4.  
[PUBMED](#) | [CROSSREF](#)