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2018

2018 , ' Reduction of F-ENO by tap water and carbonated water mouthwashes : magnitude
and time course ' Scandinavian Journal of Clinical & Laboratory Investigation , vol 78 , no.
1-2 , pp. 153-156 . DOI: 10.1080/00365513.2017.1419574

http://hdl.handle.net/10138/233094
https://doi.org/10.1080/00365513.2017.1419574

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To link to this article: https://doi.org/10.1080/00365513.2017.1419574

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Reduction of $F_{ENO}$ by tap water and carbonated water mouthwashes: magnitude and time course

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ABSTRACT
Fractional exhaled nitric oxide ($F_{ENO}$) assesses eosinophilic inflammation of the airways, but $F_{ENO}$ values are also influenced by oral nitric oxide (NO). The aim of this pilot study was to measure $F_{ENO}$ and compare the effect of two different mouthwashes on $F_{ENO}$ and analyse the duration of the effect. $F_{ENO}$ was measured in 12 randomized volunteers (healthy or asthmatic subjects) with a NIOX VERO® analyser at an expiratory flow rate of 50 mL/s. After a baseline measurement, a mouthwash was performed either with tap water or carbonated water and was measured during 20 min in 2 min intervals. The procedure was repeated with the other mouthwash. We found that both mouthwashes reduced $F_{ENO}$ immediately at the beginning compared to the baseline ($p<.001$). The carbonated water mouthwash effect lasted 12 min ($p$ ranging from $<.001$ to $<.05$). The tap water mouthwash reduced $F_{ENO}$ statistically significantly only for 2 min compared with the baseline. We conclude that a single carbonated water mouthwash can significantly reduce the oropharyngeal NO contribution during a 12 min time interval.

Introduction
The fractional concentration of exhaled nitric oxide ($F_{ENO}$) rises during eosinophilic airway inflammation and its widespread application as biomarker facilitates asthma diagnosis [1,2]. Although oral nitric oxide (NO) production contributes to $F_{ENO}$ values, a routine mouthwash remains widely unimplemented. The ATS/ERS guidelines (2005) suggest that a mouthwash may reduce oral contamination and the European Respiratory Society’s Task Force (2017) recommends a mouthwash only in physiological investigations [3,4]. However, the influence of mouthwashes continues partially unaddressed. Piirilä et al. [5] demonstrated the reduction of $F_{ENO}$ after a carbonated water mouthwash (pH 5.4–5.5). Analogously, Heijkenskold-Rentzhog et al. [6] and Zetterquist et al. [7] showed that an antiseptic chlorhexidine mouthwash (pH 8) caused a significant and long lasting decrease in $F_{ENO}$. According to Zetterquist et al., mouthwashes differ in influencing $F_{ENO}$ in magnitude and time: $F_{ENO}$ diminished minimally after a distilled water mouthwash (pH 7), on the other hand, a 10% sodium bicarbonate solution mouthwash (pH 7.85) reduced $F_{ENO}$ significantly. This contrasted to a 3% ascorbic acid solution (pH 2.5) mouthwash, which stimulated $F_{ENO}$ production [7]. Gaston et al. proposed that $F_{ENO}$ levels underlie changes in airway pH and demonstrated that a neutral buffer mouthwash (pH 7) has no effect on $F_{ENO}$ levels [8].

In Finland, a mouthwash is routinely used prior to measuring of $F_{ENO}$, either with tap water or with carbonated water. Particularly, in our laboratory in Helsinki, carbonated water is employed. Nevertheless, no previous studies have elucidated how the effect of tap water and carbonated water mouthwashes on $F_{ENO}$ values differs in magnitude and duration. The aim of this pilot study was to investigate the effect of a carbonated water mouthwash on $F_{ENO}$ as a function of time starting from a baseline and to compare the effect to a tap water mouthwash.

Methods
We recruited 12 healthy volunteers, non-smoking healthcare workers, aged 27–63 years. Three have previously had diagnosis of asthma and used inhaled corticosteroids regularly. Participants were included without further selection. The volunteers had mean (SD) height of 179 (9) cm and weight of 82 (21) kg. $F_{ENO}$ was measured during one sitting and the expiratory flow rate used was 50 mL/s. We used a NIOX VERO® analyser according to the instructions from the manufacturer [9]. The recommendations according ATS/ERS were followed [3]. The subjects refrained from drinking coffee 2 h, and from eating and drinking 1 h before the study. Strenuous exercising prior measurement was discouraged.
A testing array consisted of a baseline measurement (prior to mouthwash), followed by a mouthwash with a duration of approximately 30 s and an immediate $F_{ENO}$ measurement (time zero). A baseline measurement before each mouthwash was acquired from 2 to 4 determinations obtaining a mean. If the difference of $F_{ENO}$ values for the baseline was >1 ppb, additional exhalations were performed. The testing array continued with single determinations at intervals of 2 min until 20 min. The test was repeated with the other mouthwash about 15 min later.

The order of the mouthwashes was carried out in a manner that the first participant started with a tap water mouthwash followed by a carbonated water mouthwash. The following participant had an inverted order of the mouthwashes. The order for the next volunteer was again reverted. We continued in that fashion until all volunteers were recruited and tested.

The mouthwash consisted of rinsing the oral cavity for approximately 30 s with 100 mL either of tap water or carbonated water. The tap water used had a pH of 8.3 and the following solute concentrations: Cl$^-\ 5$ mg/L, Ca$^{2+}\ 22$ mg/L, Na$^+\ 6$ mg/L, K$^+\ 1.4$ mg/L, SO$_4^{2-}\ 10$ mg/L, and ClO$_2^-\ 0.5$ mg/L [7]. The carbonated water used for the mouthwash was a bottled drink and had an estimated pH of 5.7–5.9 and contained NaHCO$_3$, KHCO$_3$, MgCl$_2$, and CaCl$_2$ (HARTWALL VICHY ORIGINAL®, Oy Hartwall Ab, Helsinki, Finland) (Personal communication with Riitta Saleva-Sjöblom from Hartwall Ab; unreferenced). Concentration values were unavailable. We followed the ethical principles stated in the declaration of Helsinki. In addition, each participant gave a written consent and the study was approved by the ethics committee of the Helsinki University Central Hospital (HUS/1417/2016 task 13.2.01).

**Statistics**

Analysis was performed using IBM® SPSS® statistics software version 22 (IBM Corporation, Armonk, NY) and GRAPHPAD® PRISM® version 5.04 (Graphpad Software, Inc., San Diego, CA). We accepted a significance level of $\alpha = 0.05$ as statistically significant. We tested the variables with a Shapiro–Wilks test, which confirmed that they were normally distributed. Differences in the $F_{ENO}$ values between mouthwash procedures in time were tested with a general linear model (GLM) for repeated measures. $F_{ENO}$ is presented as an estimated marginal mean in ppb, 95% confidence interval (CI) [lower bound; upper bound]. The graphical material was obtained with the statistical software GRAPHPAD® PRISM® version 5.04 and graphically presented as an arithmetic mean (ppb), 95% (CI).

**Results**

$F_{ENO}$ declined significantly immediately after the tap water mouthwash from the $F_{ENO}$ baseline of 18.1 ppb (estimated marginal mean), 95% CI [13.1; 23.2] to 15.7 ppb, 95% CI [10.7; 20.7] (p < .001). After 2 min, $F_{ENO}$ (tap water) increased to 17 ppb, 95% CI [12.1; 21.9]. A significant difference was found compared to the baseline (p = .004). During the consecutive measurements (4–20 min) there were no significant differences of $F_{ENO}$ (tap water) compared to the baseline, apart from the measurement at 14 min, where $F_{ENO}$ was higher: 19.2 ppb, 95% CI [14.0; 24.4].

After the carbonated water mouthwash, $F_{ENO}$ declined immediately and significantly to 14.6 ppb, 95% CI [10.1; 19.2] (p < .001) compared with the $F_{ENO}$ baseline of 17.9 ppb (estimated marginal mean), 95% CI [12.9; 22.9]. $F_{ENO}$ (carbonated water) stayed significantly lower (p < .05) compared with the baseline during the interval of 2–12 min. At 14 min, $F_{ENO}$ (carbonated water) increased to 17.6 ppb, 95% CI [12.7; 22.6] and there was neither a statistical difference at that point nor during the consecutive measurements. Individual results are visualized in Figure 1 including the first two minutes. Individual data for the first ten minutes and baseline are included as a Supplemental file.

When comparing the differences between mouthwashes in relation to time and the baseline (pairwise comparisons), the estimated marginal mean of $F_{ENO}$ was significantly lower (p = .008) after the carbonated water mouthwash ($F_{ENO}$: 17.0 ppb, 95% CI [12.0; 22.1]) than after the tap water mouthwash ($F_{ENO}$: 18.0 ppb, 95% CI [12.9; 23.1]). When comparing the $F_{ENO}$ differences between mouthwashes (pairwise comparisons), there was no significant difference immediately after the mouthwash, i.e. at time zero (p = .083). At the 2 min measurement point, $F_{ENO}$ (carbonated water) was significantly lower than $F_{ENO}$ (tap water) (p = .03). Differences were also significant at the next time points: 4 min (p = .015), 8 min (p = .037), 12 min (p = .005), and 14 min (p = .021). Differences were not significant at 6 min (p = .141), 10 min (p = .056), 16 min (p = .736), 18 min (p = .196), and 20 min (p = .232). These main results as arithmetic means (95% CI) are visualized in Figure 2.

**Discussion**

We found that the overall effect of the carbonated water mouthwash in lowering of $F_{ENO}$ was significantly larger than the effect of the tap water mouthwash (p = .008). Immediately after the mouthwashes, both mouthwashes lowered $F_{ENO}$ on a highly significant level (p < .001) compared with the baseline, but the effect of tap water decayed rapidly. The statistically significant effect of the tap water mouthwash vanished after 2 min. The significant effect of the carbonated water mouthwash in lowering $F_{ENO}$ endured for 12 min.

When making pairwise comparisons, $F_{ENO}$ after the carbonated water mouthwash was lower than after the tap water mouthwash from time zero until 14 min, but the difference was not always significant. This might be due to the small number of subjects.

Here we demonstrate that an alkaline tap water mouthwash has a significant, but a short-lasting effect of lowering $F_{ENO}$ levels. This could be due to the alkaline pH and the low concentrations of chemically active solutes in the tap water provided by the communal water service. In Helsinki, tap water quality is regulated by law and is required to have a moderately alkaline pH value (pH > 8). Chemically active
Solute may have a low antiseptic effect, e.g. magnesium chloride and chlorine. (Personal communication with Kirsik-Marja Hiillos from Helsinki Region Environmental Services Authority HSY; unreferenced) [10]. These overall characteristics might explain the short acting effect of tap water on oral FENO. We suggest that the present study is the first one to investigate the effect of a tap water mouthwash on FENO values. Zetterquist et al. reported previously that a 10% NaHCO3 (pH 7.85) solution reduced FENO release for only 1 min [7]. In the present study, the effect of the tap water mouthwash on the FENO levels resembles that reported by Zetterquist using NaHCO3. This could possibly be explained by the alkaline pH of both solutions.

To further clarify the influence of the mouthwashes’ pH on FENO values, we may mention a previous investigation with a pH neutral phosphate buffer saline solution performed by Gaston et al. [8]. The neutral pH mouthwash showed no evident decrease in FENO. Similarly, Zetterquist et al. [7] found that a mouthwash with distilled water (neutral pH) gave a small decrease in FENO, but without reaching statistical significance.

In comparison with tap water, the carbonated water mouthwash reduced FENO values for a longer time period. The main chemical difference between the carbonated drink and tap water is the mildly acidic pH of carbonated water (5.4–5.5) which is due to carbonic acid. Additionally, carbonated water contains low levels of NaHCO3, KHCO3, MgCl2, and CaCl2. The pH of the carbonated mouthwash is slightly below the normal physiological pH of saliva, which varies between 6 and 7 [11]. The mildly acidic pH value of carbonated water seems to inhibit oral FENO production, in contrast to the stimulation and rise in FENO values observed after a highly acidic mouthwash [7]. These overall findings reinforce the hypothesis of the pH-dependent influence of mouthwashes on FENO.

It has been shown before that a fraction of FENO arises in the oropharynx [12], due to bacterial production of nitrite.

**Figure 1.** FENO (ppb) after tap water mouthwash (left side) and carbonated water mouthwash (right side). Individual FENO values (n = 12) separated by gender, during baseline (rhombi), immediately after mouthwash (squares), and after 2 min (circles).

**Figure 2.** FENO (ppb) after tap water mouthwash (rectangles) and carbonated water mouthwash (circles) in relation to time (min) and baseline. Baseline FENO obtained prior mouthwash. Data presented as arithmetic mean. Dotted lines represent the 95% CI (grey dotted line represents the 95% CI for carbonated water and black dotted line represents the 95% CI for tap water). Tested with GLM for repeated measures. Legend: *p < .001 compared with baseline, *p < .05 compared with baseline, *p < .05 pairwise comparison.
[13] and subsequent reduction of nitrite to NO [7,8]. The exact mechanism of how a carbonated mouthwash affects the $F_{ENO}$ levels requires further study, using mouthwashes with distinct chemical composition and different pH values (from acidic to neutral and alkaline). Based on the results of the present study, rinsing the oral cavity with carbonated water effectively reduces the oral $F_{ENO}$ contribution and may, thus, enable a more accurate measurement of $F_{ENO}$ arising from the lower respiratory tract. To determine if the carbonated water or tap water mouthwash procedure affects only the oral contribution to $F_{ENO}$, without affecting the alveolar concentration of NO or its alveolar diffusion, requires further investigation. Preceding investigations observed an unaffected alveolar concentration of NO through chlorhexidine mouth-washing [6].

Previously, the long-lasting effect on $F_{ENO}$ of chlorhexidine has been shown [7]. A chlorhexidine solution may be efficient in reducing oral NO [6], but it has a long-lasting effect and due to hypothetical development of bacterial resistance may be unsuitable for repeated or large-scale tests [14]. Chlorhexidine’s pronounced effect on $F_{ENO}$ probably stems from its antibacterial properties and not from the alkaline pH (pH 8). Accordingly, we did not investigate a mouthwash with chlorhexidine.

Although the number of subjects in this study was relatively small, the measurements were carefully performed and clear results were obtained. The equipment employed has an analysis duration of 1 min and 10 s and this imposed the limitation of performing only 1 determination every 2 min. When making pairwise comparisons between mouthwashes, $F_{ENO}$ after the carbonated water mouthwash was lower than after the tap water mouthwash from time zero until 14 min, but the difference was not always significant. This might be due to the small number of subjects.

We conclude that the magnitude and duration of the mouthwash’s effect on $F_{ENO}$ levels depends on the properties of the mouthwash’s solution, probably on the pH and as well on its antibacterial qualities. Ideally, a mouthwash solution should reduce oral $F_{ENO}$ production effectively, be affordable and easily accessible, and possess a pleasant taste. A carbonated water mouthwash, with a mildly acidic pH resembling that of human saliva, can effectively lower $F_{ENO}$ for a time span of approximately 12 min and suits physiological research procedures. However, these findings might also be important when considering routine clinical testing and analysing $F_{ENO}$ values near the accepted diagnostic cut-off levels, for which applying a mouthwash could affect clinical decisions.

Acknowledgements

Kirsu-Marja Hiihla and Riitta Saleva-Sjöblom for kindly providing information regarding tap water and carbonated water.

Disclosure statement

No conflicts of interest are declared by the authors.

Funding

This work was supported by the Nummela Sanatorium Foundation (PP 2015), (AS 2016), the Finnish State Funding for University-level Health Research (TYH: 2013354), The Research Foundation of the Pulmonary Diseases (PLK 2017), Tampere Tuberculosis Foundation: Eero Hämäläinen (PLK 2017), Ida Montin Foundation (PLK 2017), and Väinö and Laina Kivi Foundation (PLK 2017).

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