Oral inflammation and exhaled nitric oxide fraction: a cross-sectional study

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To the Editor:

Exhaled nitric oxide fraction (F_{ENO}) is a quantitative and noninvasive marker of respiratory inflammation and airway hyperresponsiveness [1]. Nitric oxide is formed by cells of the airway mucosa and *via* the inducible nitric oxide synthase with L-arginine as a substrate [2]. Measurements of F_{ENO} can provide an indicator of type 2 airway inflammation, and might be used for diagnosis and management of diseases, especially asthma [1], although this notion was not fully supported by a recent randomised controlled trial [3].

F_{ENO} levels have several well-established and suggested determinants, including physiological factors, such as age and sex, as well as diet, smoking, medication and infection [1]. Despite the mounting studies on F_{ENO} *per se* and its clinical application, only one small interventional study with adult asthmatic patients showed decreased F_{ENO} levels after oral care (gargle with water and brush teeth) [4]. However, the role of oral inflammation and F_{ENO} in the general population is unclear.

Periodontal diseases, like gingivitis or periodontitis, could trigger low-grade systemic inflammation [5]. In addition, aspiration of dental plaque or bacterial components and circulation of periodontal bacteria may ignite the succeeding inflammatory response [6]. Our previous research found that poor oral health is associated with declining lung function in adolescents [7]. On this line of thought, it is plausible to explore whether oral inflammation could also affect F_{ENO} levels in children and adolescents, which are generally healthy populations. Considering the existing results [4], we hypothesised that poor oral condition could result in higher F_{ENO} levels.

Our study was based on healthy, full-term German newborns that were recruited from 1995 to 1999 in the Munich study centre of the two German birth cohorts: GINIplus (German Infant Study on the Influence of a Nutritional Intervention Plus Environmental and Genetic Influences on Allergy Development) and LISA (Influence of Lifestyle Factors on the Development of the Immune System and Allergies in East and West Germany). Details of these two population-based cohorts have been previously described [8]. Relevant ethical approval and written informed consent regarding the studies were acquired beforehand.

The present cross-sectional analysis in nonasthmatics included 449 children aged 10 years and 891 adolescents aged 15 years with complete information on F_{ENO} and dental examination. F_{ENO} was measured using the NIOX MINO (Aerocrine, Sweden) as previously described [9, 10]. The dental examination has also been described previously in detail [11]. Briefly, a blunt probe was used to measure the sulcus bleeding index (SBI) and a total score was determined according to the sulcus bleeding status of each sexstant; we did not check the periodontal pockets because of the young age of the participants. The SBI ranged from 0, meaning no bleeding, to 6, referring to all sextants being affected, indicating possibly severe inflammation.

F_{ENO} data were ln-transformed (natural logarithm) to normalise the distribution. We built linear regression models to analyse the association between F_{ENO} and oral inflammation (represented by SBI categorised into 0, 1–3 and 4–6 affected sextants). The models were adjusted for study, sex, age, body mass index and parental educational level. Sensitivity analyses excluded active smokers in the 15-year group and participants with F_{ENO} levels close to the lower detection limit of the device (<10%; which corresponded

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Oral inflammation is not associated with increased F_{ENO} in nonasthmatic children and adolescents. The observed inverse association implies that gingival bleeding might decrease F_{ENO} but this needs more study to be confirmed. https://bit.ly/3IDb5nv

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to F_{ENO} 6 ppb, near the device lower detection limit of 5 ppb). In addition, we stratified the main model by three levels of high sensitivity serum C-reactive protein (CRP), a marker of systemic inflammation. All analyses were performed using R 4.1.3 (https://www.R-project.org/).

Regarding the children aged 10 years, geometric mean±SD F_{ENO} were 15.9±1.9, 13.1±2.2 and 13.3 ±2.2 ppb for the three SBI categories (0, 1–3, and 4–6, respectively), and 20.5±1.8, 19.3±1.9 and 20.9 ±1.8 ppb, respectively, for those aged 15 years.

Crude comparisons showed that F_{ENO} levels did not increase with the elevated SBI categories or oral inflammation levels either for the 10-years-old children or the 15-years-old adolescents. The adjusted models also showed that SBI categories were not correlated with increased F_{ENO} (table 1). They were even statistically significantly associated with decreased F_{ENO} in 10-year-old children. The effect estimates for the 15-year-old adolescents did not exceed the statistical significance threshold.

The association was not qualitatively affected by any of the sensitivity analyses (data not shown).

Our results were not in line with our primary hypothesis that oral inflammation may be associated with increased levels of F_{ENO}. However, we should be cautious about concluding any opposite association for several reasons. First, the observed significant inverse association between poor oral health and F_{ENO} was strongly driven by subjects with very low F_{ENO}, which was <6 ppb. The exclusion of those subjects with such F_{ENO} concentrations tuned the association down to null. Thus, the significant inverse association might be interpreted as an artefact because no plausible explanation for the finding in this subgroup of very low F_{ENO} could be identified. However, the artificial exclusion of the low-F_{ENO} subjects without any sound reasons may bias the overall association.

Second, a recent systematic review of three studies reported higher saliva nitric oxide levels in a group of adults with chronic periodontitis compared to a periodontally healthy group [12]. It is also known that F_{ENO} is lower in smokers due to a potential negative feedback effect of the nitric oxide from cigarette smoke [13, 14]. Hence, one might speculate that our observed association might be a result of a potential negative feedback mechanism of oral cavity-originated nitric oxide. However, we were unable to differentiate the nitric oxide sources and failed to verify this hypothesis.

Third, type 2 or eosinophilic inflammation is linked with F_{ENO}, while neutrophilic inflammation usually links to the production of oxidant species that may react with endogenous nitric oxide and reduce its concentration [15]. Unfortunately, our data were insufficient to differentiate between eosinophilic and neutrophilic inflammation, even though our stratified analysis for the low, medium and high CRP levels showed similar associations between oral inflammation and F_{ENO} levels across strata.

Our study exhibits two main strengths: a large sample size consisting of an almost nonsmoking population and robust associations across several sensitivity analyses. Nevertheless, the limitations of the cross-sectional design and potential selection bias cannot be neglected. The 5-ppb lower detection limit precluded us from checking the association at all F_{ENO} levels. Moreover, we were uncertain about the clinical meaning of the generally low F_{ENO}, and the generalisability of our study might be limited to children or adolescents. Hence, more longitudinal population-based studies and well-designed trials are warranted, thereby clarifying the association between oral inflammation and F_{ENO}.

Summarising the strengths and weaknesses of this study, and the attempts to interpret the findings, we conclude that oral inflammation is not associated with increased F_{ENO} levels in nonasthmatic children and

TABLE 1 Linear regression results on association between oral inflammation and exhaled nitric oxide fraction

SBI categories	10 years of age			15 years of age		
	Subjects	Means ratio (95% CI)	p-value	Subjects	Means ratio (95% CI)	p-value
0	449	Ref.		891	Ref.	
1–3	327	**0.80 (0.73–0.88)**	**<0.001**	148	0.94 (0.85–1.04)	0.232
4–6	258	**0.82 (0.73–0.92)**	**<0.001**	100	0.99 (0.89–1.12)	0.982

Adjusted for study, sex, age, body mass index and parental educational level. Means ratios were back-transformed from the natural logarithm transformation of the exhaled nitric oxide fraction. Their interpretation is similar to that of odds ratios. SBI: sulcus bleeding index. Bold indicates statistically significant results.

adolescents. In addition, we deem that more studies are needed before drawing a sound conclusion about the inverse association.

Tianyu Zhao [1,2,3,7], Elisabeth Thiering[3,4,7], Rudolf A. Jörres[1,2], Marie Standl[2,3], Jan Kühnisch [5] and Joachim Heinrich [1,2,6]

[1]Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU Munich, Munich, Germany. [2]Comprehensive Pneumology Center Munich (CPC-M), German Center for Lung Research (DZL), Munich, Germany. [3]Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany. [4]Ludwig Maximilians University of Munich, Dr von Hauner Children's Hospital, Munich, Germany. [5]Department of Operative Dentistry and Periodontology, University Hospital, Ludwig-Maximilians-Universität München, Munich, Germany. [6]Allergy and Lung Health Unit, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia. [7]These authors contributed equally.

Corresponding author: Tianyu Zhao (tianyu.zhao@med.uni-muenchen.de)

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