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Effect of face masks on salivary parameters and halitosis: Randomized controlled crossover trial

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Abstract

Background: Face masking is associated with self-perceived dry mouth and halitosis. Aim of the study was to measure the effect of different face masks on salivary parameters and halitosis.

Methods: The randomized controlled crossover clinical trial with four periods included 40 oral healthy participants using different face masks (cloth mask, surgical mask, filtering facepiece 2 [FFP2] mask) or no mask (control) for 4 h in random order. Unstimulated salivary flow rate (primary outcome) and stimulated salivary flow rate, salivary pH and buffer capacity of stimulated and unstimulated saliva (secondary outcomes, blinded), and volatile sulfur compounds (secondary outcome) were measured before and after the 4-h periods. Statistical analysis was performed by repeated measures ANOVA ($p < 0.05$).

Results: Of 40 randomized participants, 39 completed the study. Unstimulated salivary flow rate prior to face masking amounted to 0.6 ± 0.3 ml/min. Face masking had no significant effect on unstimulated salivary flow ($p = 0.550$). Face masking had also no significant effect on the other salivary parameters ($p \geq 0.518$). The concentration of volatile sulfur compounds (VSC) prior to face masking amounted to 157.3 ± 59.7 ppb. After face masking, the concentration of VSC increased slightly, but not significantly ($p = 0.055$): 168.1 ± 76.3 ppb (control), 199.3 ± 132.7 ppb (cloth masks), 188.5 ± 101.1 ppb (surgical masks), and 189.7 ± 90.1 ppb (FFP2 masks).

Conclusion: Four hours of face masking did not change the salivary flow rate, pH, and buffer capacity, and had no significant effect on VSC's levels. Wearing face masks does not seem to result in measurable side-effects on salivary parameters such as a reduced salivary flow rate or VSC's levels.

Clinical trial registration: The protocol was prospectively registered at ClinicalTrials.gov (NCT04914208) on June 4, 2021.

KEYWORDS

dry mouth, face masks, halitosis, saliva, volatile sulfur compounds

1 | INTRODUCTION

The use of face masks has been recommended during the COVID-19 pandemic for personal protection and in public health settings to prevent the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) transmission. Several systematic reviews have documented the efficacy of face masks to substantially reduce the risk of a COVID-19 infection.[1,2] Despite the recommendations by health organizations or even obligations due to national or local guidelines to use face masks in public settings, the prevalence of face masking varied significantly over time and among countries during the pandemic.[3] Some studies addressed potential barriers to face mask adherence and found, among other factors, health concerns, discomforts, political or religious beliefs, and socio-economic factors to affect face mask use.[3–5] With regard to health concerns, several studies addressed potential side effects of face masking,[6] but limited information on oral side effects is available so far.

Some studies found that the continuous use of face masks increases the perception of dry mouth[7–10] or halitosis.[10–12] Moreover, oral hygiene habits might have changed during the pandemic.[12] However, these studies have focused on self-perceived changes of oral health, potential side effects were not measured objectively so far, so that it is not known if face masking is really affecting oral health parameters. Therefore, this randomized controlled crossover study aimed to assess the effects of different face masks (cloth masks, surgical masks, filtering facepiece 2 [FFP2] masks) compared to no mask (control) on salivary flow rate, pH, and buffering capacity of unstimulated and stimulated saliva as well as on halitosis (volatile sulfur compounds [VSC]). The null hypothesis was that there is no significant effect of the different masks on salivary parameters and halitosis.

2 | MATERIALS AND METHODS

2.1 | Study design

This study was designed as a single-center, randomized, crossover, controlled clinical trial with four periods. The study was approved by the ethics committee of the University Medical Center Goettingen (no. 30/2/21) on May 18, 2021 and caried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. Reporting was performed accordingly to the Consolidated Standards of Reporting Trials (CONSORT) guidelines' extension to randomized crossover trials[13] (for CONSORT checklist and flow diagram, see Appendix S1). The protocol was prospectively registered at ClinicalTrials.gov (NCT04914208) on June 4, 2021.

2.2 | Participants

Recruitment of participants and study conduct took place at the University Medical Center Goettingen between September 2021 and April 2022. Only oral healthy, nonsmoking adults were included. Participants were excluded if they had any objections against fluoridated toothpastes. Furthermore, exemption from the obligation to wear face masks due to medical reasons, medical reasons hindering participants from refraining eating and drinking prior (except for water) or during the study visits, and hyposalivation defined as unstimulated salivary flow rate below 0.3 ml/min[14] or a stimulated salivary flow rate below 0.7 ml/min[14] were reasons for exclusion.

2.3 | Interventions

Interventions consisted of wearing different face masks for 4 h each on four different days. Three different masks were used (Figure 1): (1) cloth masks (CreaMask AP718806, two-layered: 100% cotton [140 g/m^2], 100% polyester [140 g/m^2]; ANDA Present), (2) surgical masks (NOBA-OP-Maske; NOBAMED Paul Danz), and (3) FFP2 masks (ZD-001; Zhende Medical). A 4-h period without wearing any masks served as control. Participants were instructed to refrain from consuming food associated with halitosis (e.g., garlic, hot spices, alcohol)[15] 1 day prior to each study visit. At the study days, participants should refrain from using any perfumed cosmetic products. Furthermore, they were instructed to abstain from eating or drinking prior (except for water) and during the 4-h periods.

All study visits were uniformly scheduled to start between 8 and 9 a.m. Each participant received only one intervention (i.e., wearing only one mask) per day. There was at least one free day between each study visit (washout period). All masks were supplied to the participants, and study personal (LSR) verified the correct fit of the masks at the beginning of the 4-h periods. Corrections were performed if necessary. Moreover, study participants were provided with a fluoridated toothpaste (blend-a-med classic toothpaste; Procter & Gamble) for using during the study period.

2.4 | Outcomes

Unstimulated salivary flow rate was used as primary outcome parameter. Stimulated salivary flow rate and salivary pH and buffer capacity of stimulated and unstimulated saliva as well as the amount of VSC served as secondary outcome parameters.

At each study visit, salivary samples were collected prior and after wearing the respective face masks for 4 h or the 4-h period without wearing any mask, respectively. Salivary samples were collected as described previously by Wiegand et al.[16] First, unstimulated saliva samples were collected for 5 min. Afterwards, stimulated saliva samples were collected for 5 min while chewing a paraffin pellet (MD2425; AUROSAN). The salivary flow rate (ml/min) was calculated from the weight differences of the sampling tubes prior and after saliva collection (Precisa 321; Precisa Gravimetrics).

The salivary pH was determined using a digital pH meter (HI 98100 Checker Plus pH Tester; Hanna Instruments). Prior to the assessment of the salivary buffer capacity, samples were centrifuged at 3000 rpm for 10 min (Multifuge X3R; Fisher Scientific). Afterwards,

FIGURE 1 Three different masks were used: cloth masks, surgical masks, and filtering facepiece 2 (FFP2) masks (from top to bottom)

salivary buffer capacity was determined by slightly modifying the protocol by Ericsson[17]: a total of 750 µl HCl (0.005 mol/l, pH = 2.3) was added to 250 µl saliva. After storage for 10 min at room temperature, the buffer capacity was determined by measuring the pH using a digital pH meter.

Also, the amount of VSC (ppb) released into the mouth air was measured at the beginning and in the end of each study visit (Halimeter; Interscan). Each measurement consisted of three samplings which were averaged. Prior to the study, the sensor was replaced and calibrated by an authorized service provider.

2.5 | Sample size

Sample size of this study was calculated based on the primary outcome parameter (unstimulated salivary flow rate) using the software R (www.r-project.org, version 4.0.4). At baseline, the unstimulated salivary flow rate was expected to amount to 0.8 ± 0.4 ml/min,[16] and to remain constant without wearing any face masks. After wearing masks for 4 h, a reduced salivary flow rate of 0.7 ml/min (cloth masks) or 0.6 ml/min (surgical/FFP2 masks) was expected. Irrespective of the study visits, correlation between two measurements within the same participant was expected to amount to 0.5 (compound symmetry covariance matrix). Setting $\alpha = 0.05$ and simulating the design 10 000 times demonstrated that 33 participants would be required to detect effect sizes as described above (no effect wearing no mask, 0.1 ml/min wearing cloth masks, 0.2 ml/min wearing surgical or FFP2 masks) with a power ($1 - \beta$) of 0.8. To account for potential dropouts, especially due to the pandemic situation, a total of 40 participants was anticipated.

2.6 | Randomization

All interventions were applied in random order. For each participant, the allocation sequence was generated by randomly drawing cards from a 4-card deck. Each card represented one of the interventions. Participants were enrolled and assigned to the respective interventions based on the random allocation sequence by one author (LSR).

TABLE 1 Salivary parameters and VSC among all participants ($n = 39$)

Parameter	Mask type			
	Cloth mask	Surgical mask	FFP2 mask	Control (no mask)
Flow rate (ml/min)				
Unstimulated				
Prior	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.3
After	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.3
Δ	0.1 ± 0.3	0.1 ± 0.2	0.0 ± 0.2	0.1 ± 0.2
Stimulated				
Prior	1.6 ± 0.8	1.7 ± 0.8	1.7 ± 0.9	1.7 ± 0.8
After	1.8 ± 0.8	1.8 ± 0.9	1.8 ± 0.8	1.8 ± 0.9
Δ	0.1 ± 0.3	0.0 ± 0.6	0.1 ± 0.4	0.1 ± 0.4
pH				
Unstimulated				
Prior	7.2 ± 0.5	7.2 ± 0.3	7.2 ± 0.4	7.2 ± 0.4
After	7.2 ± 0.4	7.2 ± 0.3	7.2 ± 0.4	7.2 ± 0.4
Δ	0.0 ± 0.4	0.0 ± 0.2	0.0 ± 0.3	0.0 ± 0.2
Stimulated				
Prior	7.5 ± 0.3	7.6 ± 0.3	7.5 ± 0.3	7.5 ± 0.3
After	7.5 ± 0.2	7.6 ± 0.3	7.5 ± 0.3	7.5 ± 0.3
Δ	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2
Buffer capacity				
Unstimulated				
Prior	4.2 ± 1.0	4.2 ± 1.0	4.1 ± 0.9	4.1 ± 1.1
After	4.3 ± 0.9	4.4 ± 1.0	4.2 ± 1.0	4.3 ± 1.0
Δ	0.1 ± 0.8	0.2 ± 0.6	0.1 ± 0.5	0.2 ± 0.5
Stimulated				
Prior	5.4 ± 1.3	5.6 ± 0.9	5.3 ± 1.1	5.4 ± 1.0
After	5.5 ± 1.0	5.7 ± 0.9	5.4 ± 1.1	5.6 ± 0.8
Δ	0.1 ± 0.6	0.1 ± 0.4	0.1 ± 0.7	0.2 ± 0.7
Volatile sulfur compounds (ppb)				
Prior	154.2 ± 46.1	150.5 ± 38.9	160.3 ± 79.5	164.2 ± 67.0
After	199.3 ± 132.7	188.5 ± 101.1	189.7 ± 90.1	168.1 ± 76.3
Δ	45.2 ± 115.3	38.1 ± 85.3	29.4 ± 54.8	3.9 ± 56.2

Note: Absolute values prior (baseline) and after wearing the respective mask for 4 h/a 4-h period without wearing any mask (control) are shown. Also, the absolute changes (Δ) from the respective baseline are given.

Abbreviations: FFP2, filtering facepiece 2; VSC, volatile sulfur compounds.

2.7 | Blinding

Study personal involved in the analysis of salivary samples (BR and SR) was blinded regarding the respective interventions.

2.8 | Statistical analysis

Statistical analysis was performed using the software SPSS 28.0 (IBM). For each parameter and study visit, the effect of each face mask or the 4-h period without wearing any mask was calculated as the absolute change (Δ) from the respective baseline. Using those absolute changes as observations, a repeated measures ANOVA was performed. The different masks (cloth masks, surgical masks, and FFP2 masks) and the control (no mask) were entered as within-subject factors. The level of significance was set at $\alpha = 0.05$.

3 | RESULTS

A total of 40 participants was assessed for eligibility. All participants consented and were randomized. Due to one dropout without specific

reasons, 39 participants (71.8% females, mean age: 31.8 ± 13.7) completed the trial. At baseline, unstimulated salivary flow rate amounted to 0.6 ± 0.2 ml/min (mean ± standard deviation of all groups). There were no statistically significant differences of wearing different face masks ($F[2.67,101.39] = 0.68$, $p = 0.550$). Also for the secondary outcome parameters, wearing different face masks did not show any statistically significant effect ($p \geq 0.518$).

Regarding halitosis, a small but not statistically significant ($F[2.25,85.31] = 2.90$, $p = 0.055$) increase of VSC after wearing the different face masks was detected (Table 1).

Therefore, our results fail to reject the null hypothesis.

4 | DISCUSSION

To the best of our knowledge, this is the first study aiming at quantifying frequently described oral-health related side-effects of face masking. In previous studies, participants frequently reported self-perceived dry mouth[7–10] and halitosis.[10–12] However, these self-perceived side-effects could not be verified by the quantitative measurements in the present study.

In this study, measurement of salivary parameters followed standardized protocols and revealed baseline values that are in the range of data reported for healthy adults.[18,19] Halitosis measurement was performed by detecting VSC, which are the result of the degradation of organic substrates by anaerobic bacteria. Analysis was performed using a Halimeter which can detect the total sulfur concentration, but cannot discriminate between different sulfur-containing compounds and is insensitive to dimethyl sulfide and methyl mercaptan.[20,21] The manufacturer describes the normal range between 80 and 140 ppb. Other authors give normal values of healthy adults as lying between 90 and 160 ppb.[22,23] Thus, baseline values measured in the present study were in a physiological range.

Participants were asked to wear the respective mask for 4 h based on a recent study that found the mean duration of daily face masking during the pandemic to amount to 4.7 h.[10] Moreover, several prior studies assessing potential side effects of face masking used a 4-h wearing time.[24,25]

Previous studies found that self-perception of dry mouth increases during continuous face masking. A potential explanation for this observation is a change in breathing pattern from nasal to oral breathing when using face masks.[26] Oral breathing is associated with an increased perception of oral dryness, most probably due to evaporation and mucosal drying.[27,28] The saliva flow rate itself, however, was shown to be not different between patients with oral and nasal breathing pattern.[29,30] These results are supported by the results of the present study demonstrating no effect on face masking on salivary flow rate. Consequently, pH and buffer capacity were also not changed by face masking, as they depend, among other factors, on the salivary flow rate.[31] Interestingly, a previous study found a lower salivary pH during sleeping when oral breathing was enforced by a nose clip compared to normal (i.e., nasal) breathing.[32] However, the effect of oral breathing on salivary pH was assessed over a period of about 9 h, which is distinctly longer compared to the 4-h period used in the present study.

Mouth breathing is also associated with increased levels of VSC.[28] The results of the present study indicated a trend toward a higher concentration of VSC when using the different masks. Potentially, the effect might be more pronounced when the duration of face masking would be increased over 4 h. The slightly increased VSC during face masking might be a result of evaporation, leading to reduced cleansing of the oral cavity and a shift toward more gram-negative bacteria.[28]

Besides potential changes in the breathing pattern, the use of face masks might also be associated to mental health parameters. The pandemic as such, but also the use of face masks might induce stress or anxiety symptoms,[33,34] both related to changes in salivary flow rate, dry mouth experience and/or the release of VSC.[35–37] Also, the intake of some medications (e.g., tricyclic antidepressants) might result in a reduced salivary flow rate and the perception of xerostomia.[38] However, saliva flow rate and the production of VSC are not necessarily correlated: Lima et al.[35] reported no reduction of salivary flow rate under stress conditions, but an increase of VSC, probably as a result of increased evaporation. In line with the results by Lima et al.,[35] the salivary flow rate remained stable during face masking while a slight but not statistically significant increase of VSC was noted.

For the first time, the effect of wearing face masks on objective oral health parameters was assessed in the present study. Also, different face masks which are frequently used during the ongoing COVID-19 pandemic were included. However, only participants without hyposalivation were included. The exclusion of patients with hyposalivation was made to detect even small differences in salivary parameters after the use of face masks as no previous objective data was available. As a result, the present study does not show if and how people with hyposalivation might be affected by wearing face masks. Also, halitosis was assessed by measuring the VSC's levels instead of the organoleptic assessment in order to achieve objective data. Finally, participants' breathing pattern while wearing the different face masks were not evaluated.

Future research should assess both participants' self-perception regarding dry mouth and halitosis and objective oral health parameters (i.e., salivary parameters and halitosis) at the same time.

5 | CONCLUSION

Four hours face masking did not change the salivary flow rate, pH, and buffer capacity, and had no significant effect on VSC's levels. Therefore, the use of face masks does not seem to result in measurable side-effects on oral health parameters (i.e., salivary parameters and VSC's levels).

AUTHOR CONTRIBUTIONS

Philipp Kanzow: Supervision, Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing - original

draft. **Lea-Sophie Rammert**: Investigation, Writing - review & editing. **Bianca Rohland**: Investigation, Writing - review & editing. **Sarah Barke**: Investigation, Writing - review & editing. **Marius Placzek**: Formal analysis, Validation, Writing - review & editing. **Annette Wiegand**: Conceptualization, Methodology, Writing - original draft.

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There are no funding sources to declare.

CONFLICT OF INTEREST

None to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the ethics committee of the University Medical Center Goettingen (no. 30/2/21) on May 18, 2021 and caried out in accordance with the Declaration of Helsinki.

PATIENT CONSENT STATEMENT

Written informed consent was obtained from all participants.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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