Effect of Zinc Lactate–Added Chewing Gum on Volatile Sulfur-Containing Compounds in the Oral Cavity

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ABSTRACT

Introduction: Oral Malodor is a widespread problem which can lead to social stigma and loss of self-confidence.
Methods: This controlled randomized clinical double-blinded study tested the efficacy of chewing-gums containing zinc lactate on oral volatile sulfur-containing compounds (VSC) versus placebo chewing-gum. Eligible participants had to score VSC ≥75ppb at the basal measurement. The test chewing-gum contained zinc lactate dihydrate, 0.50mg of zinc per piece; the control chewing-gum was identical but without zinc. Both chewing-gums were 1.7g. OralChroma2TM was utilized to evaluate VSC. The levels were recorded at baseline, after chewing two chewing-gums for 10 minutes; 30 minutes later; and after 1 hour and 2 hours. Data were statistically analyzed with α=0.05.
Results: 130 subjects completed the trial. The mean reduction from baseline at the end of the chewing was, respectively, 37% ($p<0.001$) in the control and 68% ($p<0.001$) in the test group, after 30 minutes it was 17% in the control ($p<0.001$) and 43% ($p<0.001$) in the test group, after 1 hour it was 6% in the control (p=NS) and 32% ($p<0.001$) in the test group, after 2 hours, it was 2% in the control (p=NS) and 15% ($p<0.001$) in the test group. The comparisons between the two groups after baseline adjustment showed a statistically significant difference in reductions at the end of chewing ($p<0.001$), after 30 minutes ($p<0.001$), after 1 hour ($p<0.001$), and after 2 hours ($p<0.05$).
Conclusion: Chewing-gums containing zinc lactate can significantly reduce the oral VSC levels immediately after consumption and for over 2 hours.

Keywords Oral Malodor, Chewing-gum, Volatile Sulfur-Containing Compounds (VSC), Zinc.

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INTRODUCTION

Oral malodor is defined as breath that is offensive to others, and it is a problem for millions of people which can lead to social stigma and loss of self-confidence (1,2,3). Its prevalence is more than 30% for moderate and around 5% for severe bad breath. It is one of the most frequent causes of access to dental visits; therefore, dentistry is giving this condition growing attention, especially by hygienists (3,4). It can be classified into intra- and extraoral due to its origin (5,6). Studies and clinicians agree that around 90% of reported cases can be attributed to intra-oral causes, and they are generally linked to periodontal disease, bacterial coating of the tongue, and some foods (5,6,7). Anaerobic bacteria present in tongue coatings are usually the primary cause of this condition (8). The tongue dorsum and its papillary structure retain a consistent amount of food debris and are easily colonized by Gram-negative anaerobes (5,8). Metabolites of these bacteria, when released in the exhaled air, are perceived as bad breath and offensive odors. They mostly include volatile sulfur-containing compounds (VSC) (2,9). Various foods such as onion, garlic, Brussels sprouts, cabbage, cauliflower, radish, spices, licorice, habits such as tobacco smoking or drinking alcohol can cause oral malodor (2). Bad breath is also common on awakening as a consequence of low salivary flow and stagnation during sleep (9). A small number of cases have extraoral origins; in these conditions, an investigation for systemic causes is important for the correct diagnosis and therapy. The traditional assessment of oral malodor was performed by smelling the exhaled air (organoleptic method) (2,3,10,11). This method is probably the most reliable in a clinical view, but it is less reproducible than a parametric measurement. Years ago, a portable detector (Halimeter) was introduced and used, but this device could not distinguish among the different types of sulfides (10,12). Today, gas chromatography is generally considered the best method to analyze, separate, and quantify the oral VSC (1,3,4,7,10,11,12). Moreover, the microbial testing of tongue coatings was proposed as a method for the assessment of oral malodor by using the BANA Strip Test, which exploits an enzyme found in Treponema denticola, Porphyromonas gingivalis, and Tannerella forsythia, three anaerobic bacteria highly associated with periodontitis and malodor (13). There is no specific treatment for oral malodor; rather, a combination of potential advice and multiple interventions has been tried, ranging from mouthwashes and toothpastes to lasers that are to be performed by the patient and the clinician (3,4). Patients

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must be informed to take regular meals and to avoid smoking, drinking, and odiferous foods. Moreover, oral hygiene by tooth brushing, flossing, and tongue cleaning is compulsory (1,2,3,4,6). Currently, oral antiseptics containing chlorhexidine, cetylpyridinium chloride, and/or zinc salts are used and proven to be effective (3,4,14). Zinc salts are reported to have a prolonged effect over three hours when administered in mouthrinses, while shorter effects have been reported with zinc-containing lozenges or chewing-gums (2,3,14,15,16,17,18,19,20). It is assumed that zinc ions form stable and non-volatile mercaptides with precursors of VSC or with the VSC directly since zinc has an affinity for sulfur (21). Zinc-containing chewing-gum was evaluated in a pilot study on 11 volunteers, and it reduced oral VSC by 45% after 5 minutes of mastication, similarly to a zinc-containing mouthrinse (16). Chewing-gum containing zinc acetate and magnolia bark extract showed a significant reduction of oral VSC levels for over one hour, and it reduced oral VSC significantly more than a control chewing-gum (19). Another trial was published on the effect of zinc lactate and magnolia bark extract added to tablets which could significantly reduce the oral VSC levels for over two hours, with a significantly better result versus the placebo at any time (22). Recently, most studies with zinc salts employed zinc lactate as the first choice to be added to chewing-gums or mouthwash (20,23,24). VSC are subjected to great variation among different hours of the day; however, a score for total VSC of more than 75 ppb is recognized by literature as detected clinical oral malodor (1,10,13). In a recent study, tablets containing zinc lactate but without magnolia bark extract showed a significant reduction of VSC from baseline at the end of the sucking period, after 1 and 2 hours (25). The present study was designed and conducted to evaluate the efficacy over time of a chewing-gum, containing only zinc lactate, on oral VSC levels versus a placebo chewing-gum. The first Null Hypothesis to test is that there is no difference for oral VSC measured in the same group between the baseline versus the VSC after mastication, and over time, the second Null Hypothesis is that there is no difference between the VSC measured at the same time, after mastication and over time, between the test and the control group.

METHODS

Study Population

200 participants were recruited in Tuscany (Italy). To enter, the subjects had to be between 18 and 60 years old and had to report no allergy to zinc salts, chewing-gum, and its ingredients. The trial was conducted by ethical principles according to the Declaration of Helsinki and Good Clinical Practice guidelines (26). All subjects had to sign a written informed consent form clearly explaining the procedures and the foods administered. Moreover, the protocol reflects the normal conduct of everyday life and therefore all ethical issues were respected. This study was also approved by the ethical committee "U.S. Investigational Review Board, Inc.," with IRB Number U.S. IRB2024PVM/01.

Study Design

This was a single-center, double-blind, clinical trial in adult volunteers. Subjects to enter the trial had to meet these inclusion criteria: be at least 18 y.o., read and sign the Informed Consent prior to the study, be in good general health based on medical and dental history, have a minimum of 20 natural teeth (8 of which posterior teeth, 4 of which upper incisors), chew chewing-gum or consume candy at least on an occasional basis, be willing to participate and able to follow the directions. In order to avoid being excluded from this trial, each potential subject must not present with any of the following: advanced periodontal disease, decayed or untreated dental sites, pathologic lesions of the oral cavity (also suspicious), full or partial dentures, fixed orthodontic appliances, xerostomia or impaired/decreased salivary function, pregnant or breastfeeding, current participation in another clinical research study, an allergy or intolerance to chewing-gum or candy ingredients, including (but not limited to) soy, phenylalanine, low-calorie artificial sweeteners, artificial colors or flavors, mint, peppermint, spearmint, milk proteins, or other ingredients, serious medical illness (immune compromised, AIDS, etc.), temporomandibular joint disorder (TMJ). All eligible participants had to avoid any professional oral hygiene, refrain from taking medicines for two weeks before the test, and not be menstruating. They received a plain toothpaste containing only amine fluoride (olaflur) (Elmex®, Gaba, Colgate-Palmolive, PL-58-100 Świdnica) to use for three days before the study and were asked to keep their daily oral hygiene. They did not have to brush their teeth and tongue, smoke, drink alcohol, coffee, or tea, or eat onion, garlic, cabbage, Brussels sprouts, cauliflower, radish, curries, or licorice for the six hours before the visit and during the test. Moreover, they were requested to drink a glass of still water 30 minutes before the test. At the beginning of the trial, they had to show a baseline oral VSC score of ≥75 ppb to start the study procedure. Each eligible subject to continue was randomly allocated according to a random table to the test or the control group. The test chewing-gum contained zinc lactate, the control chewing-gum was identical but without zinc to blind the operator and the participants from each other. All chewing-gums investigated were sugar-free, available on the market, classified as common food, and provided by the manufacturer (Perfetti Van Melle S.p.A., Lainate, MI, Italy). The OralChroma2TM (Abilit Corp., Osaka, Japan) device was used following the manufacturer's instructions to evaluate the total oral VSC. This portable gas chromatograph does not use a special carrier gas, but air and is highly sensitive, yet relatively low cost, compared with a standard gas chromatograph(10,11). Sterile single-use syringes without a needle (1ml capacity) were used to collect oral breath. Participants had their oral total VSC (H2S, CH3SH, (CH3)2S) measured before chewing (baseline), at the end of the 10 minutes of chewing two gums simultaneously, after 30 minutes, after 1 hour, and after 2 hours. They were requested to have a deep breath, to close their mouth, to set inside a thin sterile syringe, to fix it with their lips, and to wait for 30 seconds. They had

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to avoid contact between the syringe and saliva. At the end of the time, the operator pulled the syringe's piston up and down and then up again to sample 1ml of oral air. After this procedure, the operator injected the air into the OralChroma2TM. The analysis time was four minutes, allowing the follow-up of the oral VSC content at the fixed time points. This sequence was repeated at baseline, at the end of 10 minutes of chewing, then, after 30 minutes, 1 hour, and 2 hours. Since any proposed odor-reducing means should work when needed, in this study, the efficacy of this zinc lactate chewing-gum was not tested in all subjects at the same time (e.g., early morning), but around the day. The oral VSC levels vary during the day and are influenced by meals and salivary flow, therefore the testing times were however standardized in each group by the interval since the last meal and the assessment of the procedure requested before the test, obviously, the volunteers had to show a clinically measurable malodor (VSC>75ppb) before to start the trial.

Chewing–gum and dentifrice

The chewing-gums used in the trial were sugar-free and supplied by the manufacturer. They are and were available on the market under the Smint brand (Perfetti Van Melle Group B.V.1117CE Schiphol–Oost Amsterdam, The Netherlands). Both chewing-gums are a common human food and therefore they are constituted by common food ingredients and authorized food additives; moreover, they were administered in a common dosage. The test chewing-gum (1.7g) contained zinc lactate dihydrate (delivering 0.50mg of zinc per chewing-gum piece) and no additional agents reputed to be active against oral VSC; the control chewing-gum was identical in taste, shape, weight, color, and packaging but without active ingredients to blind the operator and participants from one another The dentifrice used in the trial was available over the counter, Elmex®, Gaba, Colgate-Palmolive, PL-58-100 Świdnica, it is a plain toothpaste containing only amine fluoride (olaflur), and participants were requested to maintain their regular oral hygiene procedures.

Statistical Analysis

The most comparable trial published available is one with chewing-gums containing similar ingredients (zinc acetate and magnolia bark extract) evaluated for reducing oral VSC(19). Based on the data from this research, to set the power at 80% and significance at α=0.05, for the test after 2 hours, the minimum number of participants resulted in 65 subjects for each group. The raw data were analyzed by a statistic office, independent by the authors and the manufacture that was blinded to the aim of the trial. All data were analyzed with SPSS software (IBM, Armonk, NY, USA). The comparisons within the groups were performed using a ANOVA for repeated measurements with Bonferroni correction and Tukey HSD post hoc. The differences of VSC at the end of chewing, after 30 minutes, after 1 hour, and after 2 hours between the test and the control group were compared after baseline adjustment with ANCOVA. All statistical tests of hypotheses were two-sided and employed with α=0.05.

Time	Control		Test	
	Mean	SD	Mean	SD
Baseline	119	43	123	44
After chewing	75	37	39	32
After 30′	99	35	70	32
After 1h	112	39	84	36
After 2h	117	39	105	37

Tab. 1 VSC (ppb) at various times within the groups

RESULTS

157 subjects entered the trial, but 27 were lost due to personal reasons or to their lower basal level of oral VSC (drop-off rate 17.2%); therefore, 130 volunteers completed the protocol, 65 in the test group and 65 in the control group. In the test group, they were healthy adults between the ages of 19 and 56 (mean 34±12 years), 34 women and 31 men. In the control group, they were healthy adults between the ages of 19 and 56 (mean 35±11 years), 34 women and 31 men. None reported problems referred to zinc lactate. At baseline, the VSC mean was 119±43ppb in the control group and 123±44 ppb in the test group; after 10 minutes chewing, it was 75±37 ppb in the control group and 39±32 ppb in the test group, after 30 minutes it was 99±35 ppb in the control group and 70±32 ppb in the test group, after 1 hour it was 112±39 ppb in the control group and 84±36 ppb in the test group, after 2 hours it was 117±39 ppb in the control group and 105±37 ppb in the test group. Results are summarized in Tab. I. The mean of percentage reduction from baselines at the end of chewing was 37% in the control group ($p<0.001$) and 68% in the test group ($p<0.001$), after 30 minutes it was 17% in the control group ($p<0.001$) and 43% in the test group ($p<0.001$) and after 1 hour it was 6% in the control group (p=NS) and 32% in the test group ($p<0.001$) and after 2 hours it was 2% in the control group (p=NS) and 15% in the test group ($p<0.001$). The analysis of variance showed no statistical difference between the baseline scores of the two groups. The comparisons between the test group and the control group were analyzed after the baseline adjustment as a covariate. ANCOVA showed a statistically significant difference for reductions of VSC between the test and the control chewing-gums at the end of the chewing period ($p<0.001$), after 30 minutes ($p<0.001$), after 1 hour ($p<0.001$) and after 2 hours ($p<0.05$) showing a better activity of the chewing-gums added with zinc lactate in decreasing and maintaining a lower score of total oral VSC over all the time. The acceptance of both chewing-gums was high, and no subjects reported problems related to them.

DISCUSSION

Since OralChroma2TM device can measure the three gases of VSC separately and this could be of interest in

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periodontology, in this trial we chose to follow the literature focus on the oral malodor and therefore evaluating only the sum to check it. In earlier research, authors reported the efficacy of chewing-gums containing zinc acetate and magnolia bark extract to decrease oral malodor (19). The present study was planned with a chewing-gum containing only zinc lactate, but at a higher dosage than previously evaluated. The chewing-gums employed in the present study were considered food products and, because they are not intended to cure or prevent any disease, they were tested after only one consumption episode, to determine the reduction of the bad breath immediately after their consumption and up to two hours. Mastication of chewing-gum itself may have a mechanical role in reducing bacteria or food debris by increasing saliva flow, and in some cases, it can be just as useful to reduce oral malodor as reported in a recent paper, by Zhang et al., who described its ability to reduce malodor after elective general anesthesia with endotracheal intubation with an efficacy similar to chlorhexidine (27). Therefore, to evaluate the active role of zinc lactate on the effect of VSC reduction, in the present study, we employed a placebo chewing-gum as a control. The organic salt was, however, contained at a concentration lower than those reported in past trials with mouthrinses, and similar to other research conducted with chewing gums (14,15,16,17,18,22). Previous research reported the efficacy of 2mg zinc acetate-containing chewing gum over placebo chewing-gum in diminishing oral VSC after use, however, also placebo chewing-gum showed a measurable effect, probably due to the production of stimulated saliva and the partial removal of plaque and food debris, and decreased oral VSC better than the negative control (water rinse) (16). Lately, placebo and zinc citrate-containing chewing-gum were reported to reduce VSC over time, but without a clear difference between the two gums (15). In a study with a chewing-gum containing zinc acetate and magnolia, authors reported a VSC reduction of 50.9% ($p<0.05$) from baseline at the end of chewing gum mastication, and of 27.6% ($p<0.05$) after one hour (19). Tian M. et al. tested in vivo the effect of a chewing-gum containing allyl isothiocyanate alone, and in combination with zinc salts for 3 hours and showed that the chewing-gum containing 0.1% zinc lactate and 0.01% of allyl isothiocyanate eliminated 89%, 55.5%, 48% and 24% of VSC immediately after chewing, and after 1, 2, and 3 hours, respectively (20). It is supposed that zinc lactate decreases the concentration of VSC because it comes in contact with oral fluids, releasing $Zn2+$ ions, which can form nonvolatile and stable mercaptides with VSC and their precursors due to its affinity for sulphur (14,18). A trial published on the effect of zinc lactate and magnolia incorporated into tablets, reported reductions from baselines at the end of the sucking period of 62%, one hour later of 30%, and two hours later of 18%, therefore, authors suggested that they could significantly reduce VSC levels for over two hours (22). Moreover, in a previous study with tablets containing zinc lactate but without magnolia, the mean reduction from baseline at the end of the sucking period was 67% ($p<0.001$), after 1 hour 25% ($p<0.001$) and after 2 hours 12% ($p<0.001$) (25).

The results of the present trial are consistent with those of previous studies, suggesting that an increased dose of zinc could be just as effective alone and can be a replacement for the enhancing effect due to the magnolia bark extract. The efficacy of the test chewing-gum to reduce the oral malodor is clearly shown, however, the mean VSC level did not always decrease below the clinical threshold of 75ppb. Other trial are desirable to investigate the interactions between zinc lactate administration and oral VSC reduction over time.

CONCLUSIONS

Zinc lactate added chewing-gums can significantly reduce the oral VSC levels after chewing and for over 2 hours. Moreover, the test chewing-gum reduce oral VSC significantly more than the control gum at any time, showing a better activity of the chewing-gum added with zinc lactate in decreasing and maintaining a lower score of total oral VSC over time.

Author Contributions

The authors confirm their contribution to the paper as follows: Pier Francesco Porciani, study conception, design, data collection, interpretation of results, draft manuscript preparation, and revision for intellectual content. Simone Grandini, study conception, design, supervision of the external statistical analysis, and revision of the manuscript preparation.

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Conflicts of Interest

The authors, Pier Francesco Porciani and Simone Grandini, were involved in other studies and research for Perfetti Van Melle S.p.A., which had provided funding grants for them and/or their institution.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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