



Effect of continuous sweet gustatory stimulation on salivary flow rate over time

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ABSTRACT

Objective: This study aimed to determine changes in saliva secretion and subjective taste intensity during a sustained period with continuous gustatory stimulation.

Design: Twenty-two healthy adults participated in this study. The selected taste solutions were aspartame, sucralose, and acesulfame potassium, which are nonnutritive sweeteners. The concentrations of sucralose1 and acesulfame potassium were set to show the same sweetness intensity as aspartame. Sucralose2 was twice the concentration of sucralose1. The solution was continuously fed into the oral cavity at a flow rate of 0.04 mL/min through a neck-worn precise infusion system. The salivary flow rate (g/min) after 10 min of intraoral water supply from the device was used as the baseline. Salivary flow rate, subjective taste intensity evaluated by the visual analog scale (VAS), and salivary flow rate relative to the baseline were recorded at 10, 30, 60, and 120 min after the start of the test.

Results: In the aspartame, sucralose1, and sucralose2 groups, the salivary flow rate increased significantly from 10 min to 120 min after the start of the test when compared to the rate at baseline ($p < 0.05$). The relative salivary flow rate increased and the VAS value decreased significantly over time and were affected by the time factor ($p < 0.001$, $p = 0.013$, respectively) but not by the sweetener-group factor and the interaction effects.

Conclusions: Continuous gustatory stimulation may maintain increased salivary production for a sustained period.

1. Introduction

Saliva secretion is regarded as an important factor in maintaining a healthy oral environment. Decreased saliva production has been reported to cause dysphagia, masticatory disorders, dysarthria, and dysgeusia (Graf et al., 2021); additionally, increased risks of dental caries and oral candidiasis have been reported. (Ahmad et al., 2017; Ramirez-Amador et al., 1997) In the elderly, decreased saliva production has been reported to lower the retention of denture prostheses (Inamochi et al., 2020), and thus, may lead to various oral dysfunctions and reduce the quality of life of the elderly.

Xerostomia and/or hyposalivation can be a side effect of medical prescriptions or caused by general dehydration or diabetes, even with normally functioning salivary glands (Napeñas et al., 2009). Some

conditions and diseases are known to impair salivary gland functions, such as Sjogren's syndrome and side effects of radiation therapy of the head and neck (Napeñas et al., 2009). Symptomatic treatments are adopted if hyposalivation is not improved by treatment of the causative disease or discontinuation of the drug that causes hyposalivation as a side effect. Such symptomatic treatments include oral application of a moisturizing gel (Nuchit et al., 2020) or use of artificial saliva (Łysik et al., 2019) for alleviating the symptoms of hyposalivation. Although these treatments can relieve the feeling of dry mouth, their effect is reduced by swallowing; thus, frequent application is required to maintain moisture in the oral cavity throughout the day. If the cause is Sjogren's syndrome or radiation therapy to the head and neck, oral medicines, such as cevimeline and pilocarpine, are therapeutic options. These drugs stimulate parasympathetic nerves and promote salivation;

Abbreviations: APM, aspartame; SC, sucralose; AceK, acesulfame potassium; VAS, visual analog scale.

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however, they may also induce side effects, such as sweating, headache, nausea, and vomiting (Braga et al., 2009; Wiseman & Faulds, 1995).

Previous studies have investigated treatment methods for alleviating hyposalivation by increasing the amount of saliva secreted by gustatory stimulation (Ishijima et al., 1994; Kuriwada-Satoh & Sasano, 2015). Satoh-Kuriwada et al. (2018) examined the effect of gustatory stimulation on the lip mucosa for 2 min with five basic tastes (umami, sweet, bitter, sour, and salty) and showed increased salivary secretion in the minor salivary glands of the lips. Kusakabe et al. (2020) reported that gustatory stimulation of the tongue with sweeteners, such as sucralose (SC), aspartame (APM), acesulfame potassium (AceK), rebaudioside A, and xylitol, increased salivary production. Mizuhashi et al. (2021) demonstrated increased saliva secretion using a moisturizing gel with sweet and sour tastants. In this case, saliva secretion was maintained for 30 min after application of the moisturizing gel. In contrast, Watanabe (1988) reported that salivary flow rate was highest at 5.5 – 7.7 s after gustatory stimulation with sucrose and then decreased to 50% of the maximum flow rate after 12 s. They also reported that an adaptation to taste occurred.

Regarding the adaptation, Dawes and Watanabe (1987) reported that saliva flow, which was elicited with a gustatory stimulus, declined exponentially with time. These reports showed the effect of taste on saliva secretion for a relatively short time. However, saliva secretion should be maintained for a longer period to relieve dry mouth sensation in daily life. To use the gustatory-saliva reflex against hyposalivation, we need to elucidate the changes in gustatory sensation and salivation levels by applying gustatory stimulation for a long period in a continuous and quantitative manner.

Therefore, the purpose of this study was to clarify the effect of gustatory stimulation on saliva secretion over time using a continuous and quantitative taste solution feeding system and to evaluate the strength of perceived gustatory stimulation using a visual analog scale (VAS). We hypothesized that there would be no difference in salivary secretion after continuous gustatory stimulation.

2. Materials and methods

2.1. Participants

Twenty-two healthy adults (14 males and 8 females, mean age 26.5 ± 4.0 years, range, 20 – 36 years) with no notable abnormalities in the stomatognathic system participated in this study. The participants were staff and students at the Okayama University Dental School or Okayama University Hospital. The exclusion criteria were as follows: 1) history of chemotherapy or radiation therapy, 2) on medications for hyposalivation, 3) diagnosis of zinc deficiency, or 4) saliva outflow ≤ 2 g in the Saxon test, which calculated the amount of saliva from the change in weight of the sterile gauze before and after chewing for 2 min (Kohler & Winter, 1985). This study was approved by the Research Ethics Review Board of the Okayama University Hospital (No. RIN 2108–004). Written informed consent was obtained from all study participants. All individuals participated in the trials of each gustatory stimulant.

2.2. Taste solution

Nonnutritive sweeteners with low cariogenicity were selected in consideration of future long-term supply (Zhu et al., 2021). The selected sweetening substances were APM, SC, and AceK. The reagents were added to water (natural blessings, natural water, LIFEDRINK COMPANY, Inc., Osaka, Japan) to prepare the test solutions (Mouillot et al., 2020). The concentration was determined by our preliminary experiments so that the sweetness intensities of SC and AceK were equivalent to the sweetness intensity of APM at 1 g/dL. AceK was prepared at a concentration of 1.688 g/dL. Due to the solubility of SC, two taste solutions of different concentrations, SC1 and SC2, were prepared. SC1 (0.884 g/dL) had the same sweetness intensity as AceK and APM, and

SC2 (1.769 g) was twice the concentration of SC1.

2.3. Taste solution infusion system

The taste solution was supplied to the oral cavity using a precise neck-worn infusion system (Fig. 1A). The neck-worn infusion system consisted of a micropump (RP-Q1, Aquatech, Osaka, Japan), a plastic reservoir for the solution, a controller unit (Arduino Pro Mini, SparkFun, Niwot, USA), and batteries mounted on the neck frame. The total weight of the infusion system was 249 g, including the filled reservoir. The maxillary appliance was fabricated with a 0.5 mm thermoplastic seat (DURAN®+, SCHEU-DENTAL GmbH, Iserlohn, Germany) on each participant's maxillary cast. A silicone tube 2 mm in diameter was connected from the infusion system to the buccal surface of the maxillary first molar on the maxillary appliance.

The solution was continuously fed into the oral cavity at a flow rate of 0.04 mL/min through the opening of the silicon tube. As shown in Fig. 1B, the infusion system was worn on the neck so that the participants could perform their usual daily activities with minimal annoyance.

2.4. Saliva collection

Saliva was collected using the open suction method reported by Jones et al. (2000) with a saliva suction device. During saliva collection, participants were instructed to sit on a chair in an upright position. Just prior to saliva collection, participants were instructed to swallow and clear all saliva in the mouth. During the collection of saliva, participants were instructed to lean slightly forward, look at their knees, tilt their jaw down, and not speak. Saliva was aspirated from the floor of the mouth into a 50 mL sample tube (BRAND 114822, Merck KGaA, Darmstadt, Germany) using a flexible disposable saliva ejector (Saliva Ejector φ6.5 mm, Micro Tech. Corp., Tokyo, Japan) connected to a portable suction machine (LH-930, Tokyo M.I. Co. Inc.). The salivary ejector was initially placed in the posterior buccal vestibule. The ejector was gently swept around the participant's mouth in a circular motion with one pass in the buccal vestibules and around and under the tongue at approximately 15-s intervals for 2 min. The weight of the sample tube with the saliva ejector was measured before and after the saliva sampling procedure using a precision analytical milligram scale (TE153S-DS; Sartorius AG, Göttingen, Germany), and the weight difference was calculated as the weight of the collected saliva per 2 min. The salivary flow rate (g/min) was calculated as the amount of saliva collected per min.

2.5. Experimental procedure

Each test was started 1 – 2 h after eating to avoid satiety and hunger. Participants were instructed to finish eating and brushing their teeth at least 1 h before starting the experiment; after this, eating and drinking anything other than water was prohibited until the start of the experiment. The experimental protocol for the taste solution is shown in Fig. 2. First, for baseline measurement, the neck-worn infusion system was set in place, and the maxillary appliance was worn. Water was supplied to the oral cavity for 10 min and saliva was collected during the last 2 min as described above. Next, the reservoir bottle was replaced with a taste solution bottle, and the water in the silicon tube was purged before starting the experiment. Saliva collection was performed for 2 min just before the observation times of 10, 30, 60, and 120 min. The order of the taste solutions was randomized for each participant. The examiner who retrieved the saliva samples was blinded to the taste solution used. The salivary flow rate, which represents the collected salivary weight per minute, was calculated for each test condition. The relative salivary flow rate, which is the ratio of the flow rate to that of the baseline of each participant with water, was also calculated. In our preliminary study, a similar experiment was performed with only water stimulation, and no significant change in salivary secretion was observed. Therefore, in this

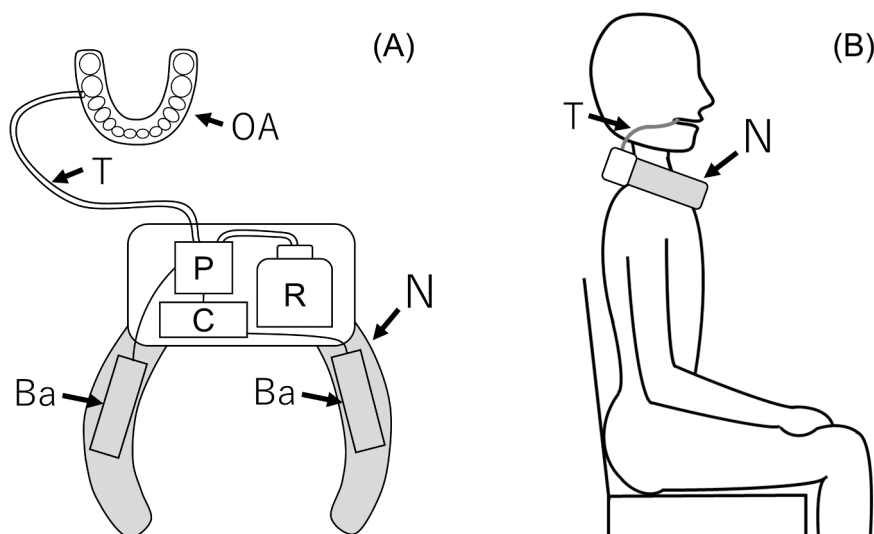


Fig. 1. Neck-worn precise infusion system (A) Schematic diagram of the neck-worn precise infusion system. N, neck frame; P, pump; C, controller unit; R, reservoir bottle for taste solution; Ba, battery; T, silicone tube; OA, oral appliance. (B) Schematic diagram of a participant wearing the infusion system.

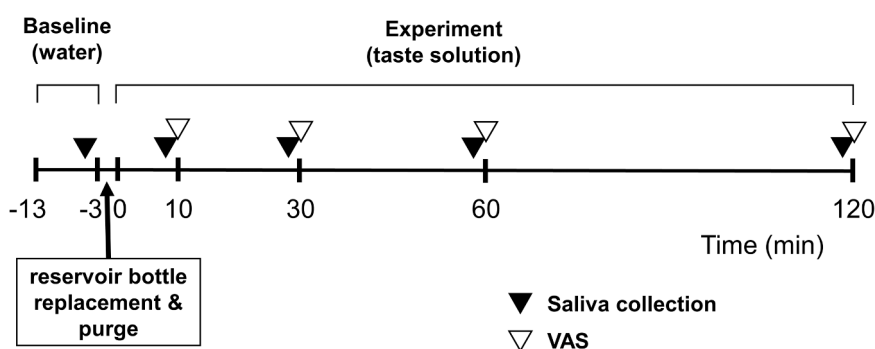


Fig. 2. Experimental protocol for a taste solution. Baseline data was taken with water from – 13 to – 3 min before time zero. From – 3 min to time zero, the reservoir bottle was replaced and the silicone tube purged. Saliva collection was performed for 2 min just before the observation time of – 3 min, 10 min, 30 min, 60 min, and 120 min. Visual Analog Scale scores were checked immediately after saliva collection at the observation times of 10–120 min.

study, the salivary flow rate by water stimulation was used as a baseline, and the relative salivary flow rate was calculated to investigate the effect of sweet stimulation. Immediately after each saliva collection, subjective taste intensity was measured using the 100-mm VAS. The VAS recorded 0 as “tasteless” and 100 as “the strongest taste imaginable.” Participants were also instructed to report whether they perceived a taste other than sweetness. After the start of the experiment, participants were instructed to go about their daily routine but were prohibited from drinking, eating, seeing foods, seeing food images, and falling asleep during the experiment. An interval of more than one week was set between the trials for each taste solution.

2.6. Statistics

Normality was verified using the Shapiro–Wilk test and homoscedasticity using Levene’s test. The Friedman test was performed for comparison of changes of the salivary flow rate over time of each gustatory stimulant, and Scheffe’s pair comparison was used as a post-test. Differences in the relative salivary flow and the VAS values according to the sweetener-group and the time of stimulation were analyzed by using the two-way ANOVA and a post hoc test (Bonferroni method). For comparing the VAS values, salivary flow rate, and relative salivary flow rate between the group that perceived non-sweetness and the group that perceived only sweetness, the Mann–Whitney U test was performed. The significance level was set to 5%, and Bell Curve for Excel 2.15

(Social Survey Research Information Co., Ltd., Tokyo, Japan) was used for statistical analysis.

3. Results

By eliminating invalid data where participants could not suppress unintended swallowing during saliva collection, valid data were collected from 21 participants for APM, 22 for SC1, 21 for SC2, and 20 for AceK. Fig. 3 shows the change in the salivary flow rate over time from the baseline to the end of the 120 min experiment for each taste solution. According to the Friedman test and Scheffe’s pair comparison, in APM, SC1, and SC2, the salivary flow rate increased significantly from 10 min to 120 min after the start of the test when compared to the baseline (APM:10 min, $p = 0.03$; 30 min, $p = 0.006$; 60 min, $p < 0.001$; 120 min, $p < 0.001$) (SC1:10 min, $p = 0.019$; 30 min, $p < 0.001$; 60 min, $p < 0.001$; 120 min, $p < 0.001$) (SC2:10 min, $p = 0.008$; 30 min, $p = 0.002$; 60 min, $p < 0.001$; 120 min, $p < 0.001$). In AceK, the salivary flow rate increased significantly from 30 min to 120 min after the start of the test when compared to the baseline (AceK:30 min, $p = 0.008$; 60 min, $p = 0.02$; 120 min, $p < 0.001$). Fig. 4 shows the transition of the mean value of the relative salivary flow rate for each taste solution. According to the two-way ANOVA performed for analyzing the relative salivary flow, a significant difference in the time factor ($p < 0.001$) was noted between the groups. We did not find any significant difference in the sweetener-group factor ($p = 0.270$) and the

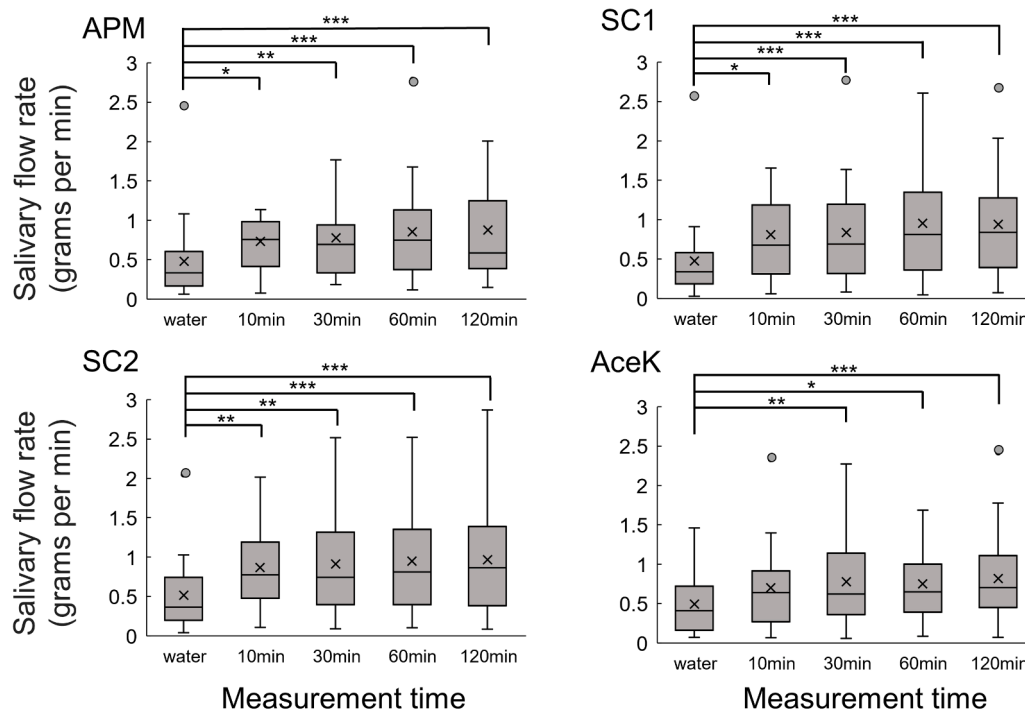


Fig. 3. Effect of taste solution on salivary flow rate. The box represents the median $\pm$ 25 percentile values and the vertical bar indicates the minimum and maximum values. The "x" mark indicates the mean value and the bar in the box indicates the median value. (APM: $n = 21$, SC1: $n = 22$, SC2: $n = 21$, AceK: $n = 20$) (*denotes $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$: Friedman's test and Scheffe's pair comparison).

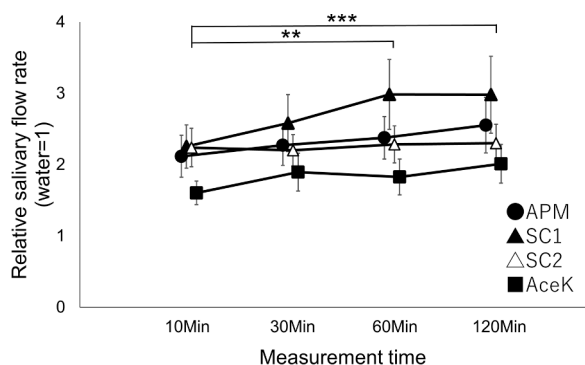


Fig. 4. Effect of taste solution on the relative salivary flow rate. Each dot represents the mean value for each condition. The vertical bar indicates standard error. (APM: $n = 21$, SC1: $n = 22$, SC2: $n = 21$, AceK: $n = 20$) (the punctuation mark denotes significant difference between the measuring time: ** $p < 0.01$, *** $p < 0.001$, two-way ANOVA and Bonferroni method).

Table 1
VAS scores for four taste solutions at each measuring time.

Taste solution	VAS score (mean $\pm$ SE)			
	10 min	30 min	60 min	120 min
APM	44.4 $\pm$ 4.3	46.2 $\pm$ 3.9	41.0 $\pm$ 3.8	40.9 $\pm$ 3.4
SC1	49.0 $\pm$ 4.3	48.0 $\pm$ 3.8	46.6 $\pm$ 3.5	44.2 $\pm$ 3.9
SC2	55.2 $\pm$ 4.5	50.0 $\pm$ 3.8	47.4 $\pm$ 4.0	44.7 $\pm$ 4.3
AceK	40.3 $\pm$ 4.9	37.9 $\pm$ 4.9	37.7 $\pm$ 4.3	40.0 $\pm$ 5.0
Total	47.3 $\pm$ 2.3	45.6 $\pm$ 2.1	43.3 $\pm$ 2.0	42.5 $\pm$ 2.1

*denotes significant difference between measuring time ($p < 0.05$). VAS, visual analog scale; APM, aspartame; SC, sucralose; AceK, acesulfame potassium; SE, standard error.

interaction effects ($p = 0.327$) between the groups. In addition, significant differences were observed between the 10 min and 60 min time-points ($p = 0.0069$) and the 10 min and 120 min time-points ($p < 0.001$) by the Bonferroni method. Table 1 shows the mean and standard error of the VAS values for each taste solution. According to the two-way ANOVA performed for analyzing the VAS values, a significant difference in the time factor ($p = 0.013$) was observed between the groups. We did not find any significant differences in the sweetener-group factor ($p = 0.218$) and the interaction effects ($p = 0.559$) among the groups. In addition, significant differences were observed between the 10 min and 120 min time-points ($p = 0.0184$) by the Bonferroni method. The number of participants who reported tastes other than sweetness was 0 for APM, 0 for SC1, 10 for SC2, and 7 for AceK. The taste reported from these participants was bitter. There were only two who reported bitterness for both SC2 and AceK. Therefore, the data for SC2 and AceK were further analyzed by dividing them into a group that reported bitterness (bitterness group: B-group) and a group that did not report bitterness (non-bitterness group: NB-group). Fig. 5 shows the relationship between the VAS value and the relative saliva flow rate and the relationship between the VAS value and the salivary flow rate in B-group and NB-group for SC2. The Mann–Whitney U test was performed for investigating the VAS values, the relative saliva flow rate, and the salivary flow rate between the B- and NB-group. In SC2, there was no significant difference in the VAS value ($p = 0.865$) or salivary secretion ($p = 0.437$) between the B- and NB-groups. However, the relative salivary flow rate in SC2 was significantly lower ($p < 0.001$) in B-group than in NB-group. These findings suggest that SC2 may be perceived as bitter regardless of the individual amount of saliva secretion, and, when it is perceived as bitterness, the saliva secretion-promoting effect is significantly low. Fig. 6 shows the relationship between the VAS value and the relative saliva flow rate and the relationship between the VAS value and the salivary flow rate in the B- and NB-groups for AceK. The Mann–Whitney U test performed for investigating the VAS values, the relative saliva flow rate, and the salivary flow rate between the B- and NB-group. In AceK, the VAS score of the B-group was significantly higher ($p < 0.001$) than that of NB-group. For AceK, the

SC2

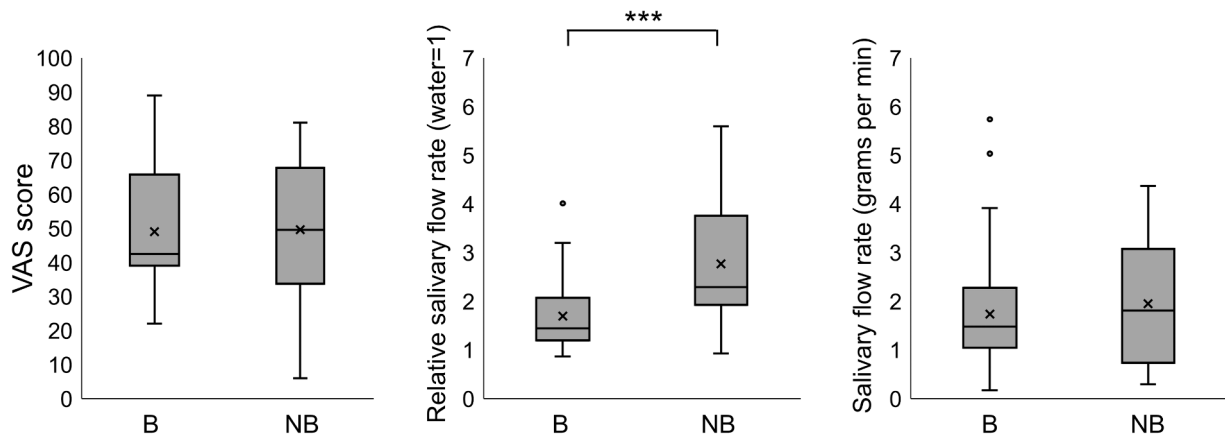


Fig. 5. Visual Analogue Scale (VAS) scores and the relative salivary flow rate in the bitterness group (B-group, $n = 10$) and non-bitterness group (NB-group, $n = 11$) for sucralose (SC2). The box represents the median $\pm$ 25 percentile values and the vertical bar indicates the minimum and maximum values. The “x” mark indicates the mean value and the bar in the box indicates the median value. (***)denotes $p < 0.001$, Mann–Whitney U test).

AceK

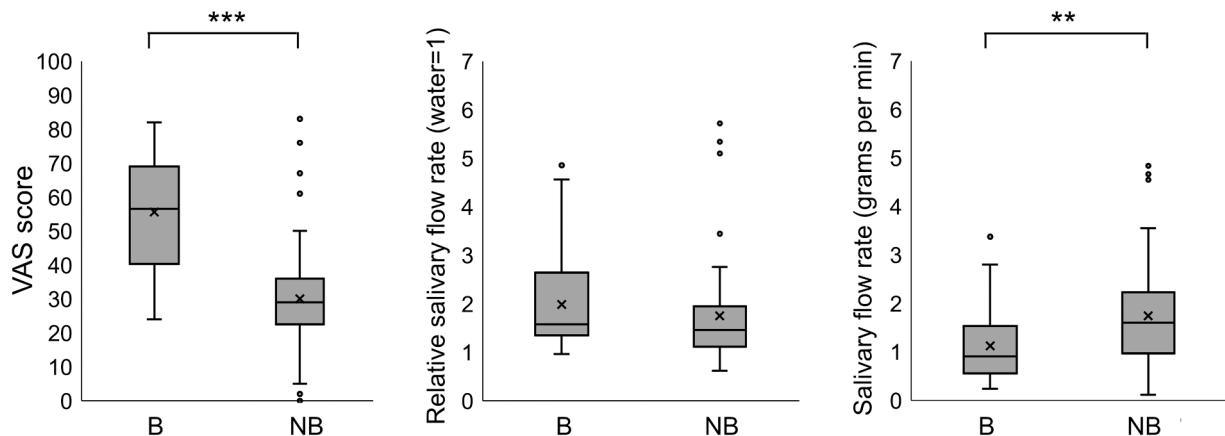


Fig. 6. Visual Analogue Scale (VAS) scores and the relative salivary flow rate in the bitterness group (B-group, $n = 7$) and non-bitterness group (NB-group, $n = 13$) for acesulfame potassium (AceK). The box represents the median $\pm$ 25 percentile values and the vertical bar indicates the minimum and maximum values. The “x” mark indicates the mean value and the bar in the box indicates the median value. (***)denotes $p < 0.001$, Mann–Whitney U test).

salivary flow rate observed in B-group was significantly lower than that in NB-group ($p = 0.0063$). However, there was no significant difference in the relative salivary flow rate of AceK between B-group and NB-group ($P = 0.155$).

4. Discussion

In this study, we examined the effects of sustained gustatory stimulation on salivation over a relatively long period (2 h). A prolonged acceleration of salivary flow rate by sustained gustatory stimulation was observed in this study; thus, the null hypothesis set was rejected. Extensive knowledge of the effect of sustained and quantitatively controlled gustatory stimulation on salivary flow would be important as a scientific basis to develop modalities for the treatment of hyposalivation, such as sustained-release drugs. Most previous studies have reported only a short-lasting increase of the salivary production by stimulating the gustatory-saliva reflex. Mizuhashi et al. (2021) reported an increase in salivary flow in healthy participants using an oral moisturizing gel containing sorbitol and xylitol for gustatory stimulation. However, the observation time was 30 min. To address the feeling of dryness in daily life, it would be best to continuously provide gustatory

stimulation without restraint and maintain a moist condition for a longer period. In this study, continuous and quantitative gustatory stimulation under daily-life conditions was made possible by fabricating a system that could supply the taste solution without restraint. Although we evaluated a limited number of tastants in this study, further investigations are possible with this system to obtain the scientific evidence necessary to develop new therapeutic methods against hyposalivation.

Regarding the selection of sweeteners, nonnutritive sweeteners with low cariogenicity were selected in consideration of future long-term supply (Zhu et al., 2021). In addition, low-intensity sweeteners were excluded (e.g., sugars, alcohols, etc.) as it was essential that the sweetener be perceived even in minimal amounts in the solution (0.04 mL/min). In previous studies, many gustatory stimulants have been tested for saliva flow stimulation. Among the five basic tastes, sourness, umami, and saltiness have been shown to have a higher efficiency for saliva secretion than sweetness and bitterness (Hodson & Linden, 2006; Kono et al., 2018; Satoh-Kuriwada et al., 2018). Our final aim was to use long term gustatory stimulation as a clinical treatment for dry-mouth patients. Therefore, the use of sour tastants (acids) was excluded from our selection because acids may demineralize of the

teeth. In addition, the use of bitter tastants was excluded because of the potential discomfort of bitter taste. Regarding saltiness (NaCl), continuous application of NaCl would have an undesirable effect on the participants' systemic health, especially in those with hypertension. However, we were able to use artificial sweeteners that do not induce dental caries and can possibly be used in patients with diabetes.

The concentrations of the APM, SC1, and AceK taste solutions were adjusted to induce equivalent sweetness according to the findings of our preliminary experiment. The VAS evaluation for APM, SC1, and AceK ranged from 40 – 50. However, AceK tended to have lower VAS values than the other sweeteners. It has been reported that APM and SC have higher persistency of sweetness than AceK (Tan et al., 2019), which may affect the VAS values of APM, SC, and AceK in our experiments using 120 min stimulation. In our preliminary experiment, sweet taste intensity was evaluated 60 s after the direct application of taste solution on the tongue surface. This time course differed from the continuous stimulation of tastants for 120 min in subsequent experiments. Lower persistency of AceK sweetness may contribute to the lower VAS value of sweetness in its long-term continuous application. However, this point should be further investigated.

In this study, several processes that promote salivary flow dynamics were observed. SC1 and SC2 showed different secretory-promoting effects. Although the initial secretion amount of low-concentration SC1 was equal to that of high-concentration SC2, SC1 tended to increase saliva secretion gradually over time and showed the highest value at 120 min. This contradicts the general physiological process of adaptation. One possibility is the accumulation of sweet substances in the oral cavity due to long-term continuous administration, albeit at very small doses. In a study by Kusakabe et al. (2020), granulated sugar, AceK, APM, SC, and xylitol were used as gustatory stimulants, and they found correlations among the response properties of sweetness receptors, sweetness intensity, and salivation. However, in our study, the amount of saliva secretion tended to increase with time, although the VAS value tended to decrease slightly with time. This result suggested that the effect of the intensity of the subjective gustatory perception on continuous salivary secretion is unclear. We do not yet know the reason for this phenomenon; therefore, further research is needed, especially regarding the long-term administration of low-concentration sweeteners. If the amount of saliva secretion increases without a perception of sweetness, more comfortable modalities may be incorporated into a patient's daily life. Therefore, it is important to seek the minimum concentration of taste materials, such as SC, which increases salivary flow secretion while perceiving less taste for a much longer time course than 120 min.

The difference in salivation-inducing effects among sweeteners may be due to the taste characteristics of the artificial sweeteners. As observed in this study, some artificial sweeteners have a bitter taste. Antenucci and Hayes (2015) reported that bitterness increases for AceK and SC with concentration. Eventually, the sweetness intensity of SC was almost equal to that of bitterness at high concentrations, and the bitterness of AceK surpassed perceived sweetness at high concentrations. Their data also showed that startup concentration of bitterness perception for AceK is close to that of sweetness, whereas the startup concentration of bitterness perception for SC is much higher than the startup concentration of sweetness for SC. Many reports have demonstrated that bitter taste itself affects salivary secretion. Although bitterness promotes saliva secretion, its effect is lower than that of sweetness. Bitterness has been reported to have the least effect on salivary secretion among the four basic tastes (Hodson & Linden, 2006). Satoh-Kuriwada et al. (2018) suggested that bitter-induced salivary secretion may be caused by a mechanism different from that of parasympathetic nerve-mediated saliva secretion, i.e., aversion or potential resistance. In addition, it has been reported that the preferred taste in the taste test affects salivation more effectively than non-preferred tastes (Dietsch et al., 2018). In the case of AceK, there was no significant difference in the salivary secretion-promoting effects between the B-group and NB-group (Fig. 6). However, the amount of salivary secretion in the

B-group was significantly lower than that in the NB-group (Fig. 6). This may suggest that the salivary secretion-promoting effect of AceK would not be affected by bitter taste perception, but bitter perception may correlate with the amount of saliva secretion. Further investigations are necessary. In contrast, with SC2, there was no significant difference in saliva secretion between B-group and NB-group, and the relative salivary secretion ratio in B-group was significantly lower than that in NB-group. Therefore, the perception of bitterness from SC2 may not be associated with the initial amount of salivary secretion; however, the salivary secretion-promoting effect seemed to be lower in these participants. Considering that no participant complained of bitterness at a lower concentration of SC (SC1), the SC1 concentration may be a concentration that could be used to promote salivation for a long period. Although the bitter tastes induced by SC2 and AceK themselves differed significantly in the participant reports, no further information on the difference in bitterness could be gained in this study. Twenty-five bitterness receptors have been identified in humans. (Behrens & Meyerhof, 2006; Maehashi & Huang, 2009) In the present study, 10 participants reported bitterness for SC2 and 7 for AceK. However, only two participants reported bitterness, both for SC2 and AceK. Therefore, these two gustatory stimulants may work on different bitterness receptors. Further studies on such differences in the taste characteristics of artificial sweeteners are needed.

4.1. Limitations

The use of a small number of taste solutions was a limitation of this study. The small sample size is another limitation. Significant differences were not observed in VAS values between SC1 and SC2 for all time points. Therefore, evaluation of the dose-response relationship might not be sufficient for only observing these two concentrations. An increased number of participants using various taste solution conditions is expected in future studies. The results of the present study revealed that continuous gustatory stimulation maintained increased salivary production for up to 120 min. Therefore, it is suggested that continuous gustatory stimulation has the potential to be applied as a modality for treating hyposalivation. However, we need to verify whether these sweeteners affect patients with xerostomia and/or hyposalivation similar to their effect in healthy adults. We are also considering evaluating sustained-release tablets and film sheets as they are more accessible clinically. It is also suggested that there are cases in which the subjective sweetness evaluation and the magnitude of the promotion of salivary flow rate do not necessarily correlate during continuous gustatory stimulation; more detailed features of continuous gustatory stimulation should be explored in future studies.

5. Conclusion

The results of the present study suggest that continuous gustatory stimulation can maintain increased salivary production for a sustained period. Sustained gustatory stimulation using an artificial sweetener may be a future modality for relieving hyposalivation.

CRedit authorship contribution statement

Ranko Yamada: Conceptualization, Writing – original draft. **Yuki Tanaka:** Conceptualization, Writing – review & editing. **Hikaru Sugimoto:** Conceptualization, Writing – review & editing. **Naoki Kodama:** Writing – review & editing. **Ryusuke Yoshida:** Writing – review & editing. **Shogo Minagi:** Supervision, Writing – review & editing. We confirm that the manuscript has been read and approved by all named authors, all the authors contributed with the present study.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Ahmad, M. S., Bhayat, A., Zafar, M. S., & Al-Samadani, K. H. (2017). The impact of hyposalivation on quality of life (QoL) and oral health in the aging population of Al Madinah Al Munawwarah. *International Journal of Environmental Research and Public Health*, 14, 445. <https://doi.org/10.3390/ijerph14040445>
- Antenucci, R. G., & Hayes, J. E. (2015). Nonnutritive sweeteners are not supernormal stimuli. *International Journal of Obesity*, 39, 254–259. <https://doi.org/10.1038/ijo.2014.109>
- Behrens, M., & Meyerhof, W. (2006). Bitter taste receptors and human bitter taste perception. *Cellular and Molecular Life Sciences*, 63, 1501–1509. <https://doi.org/10.1007/s00018-006-6113-8>
- Braga, M. A., Tarzia, O., Bergamaschi, C. C., Santos, F. A., Andrade, E. D., & Groppo, F. C. (2009). Comparison of the effects of pilocarpine and cevimeline on salivary flow. *International Journal of Dental Hygiene*, 7, 126–130. <https://doi.org/10.1111/j.1601-5037.2008.00326.x>
- Dawes, C., & Watanabe, S. (1987). The effect of taste adaptation on salivary flow rate and salivary sugar clearance. *Journal of Dental Research*, 66, 740–744. <https://doi.org/10.1177/00220345870660030701>
- Dietsch, A. M., A Pelletier, C., & Solomon, N. P. (2018). Saliva production and enjoyment of real-food flavors in people with and without dysphagia and/or xerostomia. *Dysphagia*, 33, 803–808. <https://doi.org/10.1007/s00455-018-9905-8>
- Graf, S., Kirschstein, L., Knopf, A., Mansour, N., Jeleff-Wölfler, O., Buchberger, A., & Hofauer, B. (2021). Systematic evaluation of laryngeal impairment in Sjögren's syndrome. *European Archives of Oto-rhino-laryngology*, 278, 2421–2428. <https://doi.org/10.1007/s00405-021-06746-0>
- Hodson, N. A., & Linden, R. W. (2006). The effect of monosodium glutamate on parotid salivary flow in comparison to the response to representatives of the other four basic tastes. *Physiology & Behavior*, 89, 711–717. <https://doi.org/10.1016/j.physbeh.2006.08.011>
- Inamochi, Y., Fueki, K., Matsuyama, Y., Yoshida-Kohno, E., Fujiwara, T., & Wakabayashi, N. (2020). Does oral dryness influence pressure pain sensitivity in the oral mucosa of removable denture wearers? *Clinical Oral Investigations*, 24, 2603–2609. <https://doi.org/10.1007/s00784-019-03118-1>
- Ishijima, T., Hirose, T., Hirai, T., Aoki, S., Yukino, E., & Watanabe, S. (1994). Clinical evaluation of saliva stimulating agent. *Nihon Hotetsu Shika Gakkai Zasshi*, 38, 636–643.
- Jones, J. M., Watkins, C. A., Hand, J. S., Warren, J. J., & Cowen, H. J. (2000). Comparison of three salivary flow rate assessment methods in an elderly population. *Community Dentistry and Oral Epidemiology*, 28, 177–184. <https://doi.org/10.1034/j.1600-0528.2000.280303.x>
- Kohler, P. F., & Winter, M. E. (1985). A quantitative test for xerostomia. The Saxon test, an oral equivalent of the Schirmer test. *Arthritis and Rheumatism*, 28, 1128–1132. <https://doi.org/10.1002/art.1780281008>
- Kono, Y., Kubota, A., Taira, M., Katsuyama, N., & Sugimoto, K. (2018). Effects of oral stimulation with capsaicin on salivary secretion and neural activities in the autonomic system and the brain. *Journal of Dental Sciences*, 13, 116–123. <https://doi.org/10.1016/j.jds.2017.08.007>
- Kuriwada-Satoh, S., & Sasano, T. (2015). New remedy for dry mouth using the gustatory-salivary reflex. *Yakugaku Zasshi*, 135, 783–787. <https://doi.org/10.1248/yakushi.14-00250-4>
- Kusakabe, Y., Shindo, Y., Kawai, T., Maeda-Yamamoto, M., & Wada, Y. (2020). Relationships between the response of the sweet taste receptor, salivation toward sweeteners, and sweetness intensity. *Food Science & Nutrition*, 9, 719–727. <https://doi.org/10.1002/fsn3.2036>
- Lysik, D., Niemirowicz-Laskowska, K., Bucki, R., Tokajuk, G., & Mystkowska, J. (2019). Artificial saliva: challenges and future perspectives for the treatment of xerostomia. *International Journal of Molecular Sciences*, 20, 3199. <https://doi.org/10.3390/ijms20133199>
- Maehashi, K., & Huang, L. (2009). Bitter peptides and bitter taste receptors. *Cellular and Molecular Life Sciences*, 66, 1661–1671. <https://doi.org/10.1007/s00018-009-8755-9>
- Mizuhashi, F., Koide, K., & Toya, S. (2021). Effectiveness of oral moisturizing gel and flavor on oral moisture and saliva volume: A clinical study. *The Journal of Prosthetic Dentistry*, 125, 767–771. <https://doi.org/10.1016/j.prosdent.2020.02.031>
- Mouillot, T., Parise, A., Greco, C., Barthet, S., Brindisi, M. C., Penicaud, L., ... Jacquin-Piquet, A. (2020). Differential cerebral gustatory responses to sucrose, aspartame, and stevia using gustatory evoked potentials in humans. *Nutrients*, 12, 322. <https://doi.org/10.3390/nu12020322>
- Napenas, J. J., Brennan, M. T., & Fox, P. C. (2009). Diagnosis and treatment of xerostomia (dry mouth). *Odontology*, 97, 76–83. <https://doi.org/10.1007/s10266-008-0099-7>
- Nuchit, S., Lam-Ubol, A., Paemuang, W., Talungchit, S., Chokchaitam, O., Mungkung, O. O., ... Trachootham, D. (2020). Alleviation of dry mouth by saliva substitutes improved swallowing ability and clinical nutritional status of post-radiotherapy head and neck cancer patients: A randomized controlled trial. *Supportive Care in Cancer*, 28, 2817–2828. <https://doi.org/10.1007/s00520-019-05132-1>
- Ramirez-Amador, V., Silverman, S., Jr, Mayer, P., Tyler, M., & Quivey, J. (1997). Candidal colonization and oral candidiasis in patients undergoing oral and pharyngeal radiation therapy. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, 84, 149–153. [https://doi.org/10.1016/s1079-2104\(97\)90061-5](https://doi.org/10.1016/s1079-2104(97)90061-5)
- Satoh-Kuriwada, S., Shoji, N., Miyake, H., Watanabe, C., & Sasano, T. (2018). Effects and mechanisms of tastants on the gustatory-salivary reflex in human minor salivary glands. *BioMed Research International*, 2018, 3847075. <https://doi.org/10.1155/2018/3847075>
- Tan, V., Wee, M., Tomic, O., & Forde, C. G. (2019). Temporal sweetness and side tastes profiles of 16 sweeteners using temporal check-all-that-apply (TCATA). *Food Research International*, 121, 39–47. <https://doi.org/10.1016/j.foodres.2019.03.019>
- Watanabe, S. (1988). The effect of taste adaptation on the parotid. *The Japanese Journal of Pedodontics*, 26, 769–774.
- Wiseman, L. R., & Faulds, D. (1995). Oral pilocarpine: A review of its pharmacological properties and clinical potential in xerostomia. *Drugs*, 49, 143–155. <https://doi.org/10.2165/00003495-199549010-00010>
- Zhu, J., Liu, J., Li, Z., Xi, R., Li, Y., Peng, X., ... Zhou, X. (2021). The Effects of nonnutritive sweeteners on the cariogenic potential of oral microbiome. *BioMed Research International*, 2021, 9967035. <https://doi.org/10.1155/2021/9967035>