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Note

Effect of Oral Stimulation with Ginger (*Zingiber officinale* Roscoe) Juice on Salivary Secretion

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ABSTRACT

This study demonstrated the effect of oral stimulation with ginger juice (GJ) on salivary secretion. Compared with distilled water (DW), oral stimulation with GJ increased total salivary secretion. Moreover, the total salivary secretion induced by GJ was higher than those by citric acid (CA) and monosodium L-glutamate (MSG). Markers of autonomic nervous activity (RR interval, standard deviation of RR intervals, and root mean square of successive differences between RR intervals) were not different between the solutions. The pungency components of GJ included 6-gingerol and 6-shogaol, with 6-gingerol being the major component. Thus, the increase in salivary secretion by oral stimulation with GJ may have been induced by pungency components, such as 6-gingerol and/or 6-shogaol. The results indicate that GJ helps increase salivary secretion and maintain oral health.

Key words: ginger, saliva, pungency, 6-gingerol, heart rate variability (HRV)

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is a plant whose rhizome is used as a seasoning spice in Western, Chinese, and Japanese cuisines. Ginger has a pungent taste, which is associated with pungency components, such as gingerols, shogaols, paradols, zingerone, gingerdiones, and gingerdiols¹⁾. Specifically, 6-gingerol is the major pungency component in ginger with diverse bioactivities, such as antioxidant, anti-inflammatory, and antibacterial activities^{1)~6)}. Ginger, its extract, and pungency components can induce anti-obesity and exert weight-lowering effects by activating the sympathetic nervous system⁷⁾. Its active components appear to exert an agonistic effect on transient receptor potential vanilloid type 1 (TRPV1), a temperature-sensitive receptor, activate the sympathetic nerve system, and release norepinephrine⁷⁾⁸⁾. Ginger and its pungency components have received considerable attention in health-related studies.

Saliva has various important functions, such as the digestion of starch by α -amylase, assistance with taste, antibacterial defense, buffering, protection, lubrication,

and tissue repair^{9)~11)}. Thus, saliva is important for oral and systemic health. Salivary secretion is regulated by the autonomic nervous system¹²⁾¹³⁾. Dominance of the parasympathetic nervous system induces the secretion of a large volume of saliva, along with water and ions. Conversely, dominance of the sympathetic nervous system induces the secretion of protein (e.g., enzymes, secretory immunoglobulin A, and mucin).

Recently, increasing salivary secretion by oral stimulation with taste components is empirically well established^{14)~16)} and is a promising approach to improving xerostomia and maintaining oral and general health. For example, oral stimulation with taste components, such as monosodium L-glutamate (MSG), citric acid (CA), sucrose, sodium chloride, and quinine, stimulates salivary secretion^{14)~16)}. Oral stimulation with pungency components, such as capsaicin, also induces salivary secretion more effectively than basic taste components. Specifically, capsaicin can induce the activation of the sympathetic nervous system¹⁷⁾¹⁸⁾. 6-Gingerol, which has a similar structure to capsaicin, can bind to TRPV1¹⁹⁾. Thus, oral stimulation with ginger components may induce salivary secretion with efficacy

comparable to that of capsaicin. Ginger consumption can increase salivary secretion in cattle²⁰). Kan et al. demonstrated that intraperitoneal injection of ginger extracts (70% methanol, 80% ethanol, and dimethyl sulfoxide extracts) and 6-shogaol for 3 weeks increased salivary flow in mice²¹). Patients who underwent radiotherapy who took methanolic ginger extract for 2 weeks reported increased salivary secretion and improved xerostomia symptoms²²). Oral stimulation with ginger ethanol extract spray was found to increase salivary secretion in patients with type II diabetes²³). Previous studies involving humans have demonstrated increased salivary secretions using ginger extracts^{22,23}). However, the effect of oral stimulation using ginger and/or ginger juice (GJ) on salivary secretion, which can be used daily, has not been investigated. Therefore, in this study, we sought to address this research gap and investigate the effect of oral stimulation with GJ on salivary secretion.

MATERIALS AND METHODS

Preparation of GJ and taste component solutions

Ginger rhizomes were purchased from the CONSUMERS CO-OPERATIVE KOBE, a local supermarket in Kobe City, Hyogo Prefecture, Japan, between November and December 2023. All samples were washed with water and peeled. Excess water was removed using a paper towel. GJ was prepared by extracting ginger samples using a juicer (undiluted juice). The undiluted GJ concentrate was too pungent and could not be ingested, so it was diluted 10 times with distilled water (DW; diluted juice). This diluted juice was then used as an oral stimulation solution. On each experimental day, ginger rhizomes were purchased, and undiluted and diluted juices were prepared. The control solution was DW.

The characteristics of salivary secretion induced by oral stimulation with GJ were evaluated and compared with those induced with taste compound solutions based on CA and MSG. Previous studies have shown that CA and MSG solutions can increase salivary secretion^{14)~17)}. The CA (10 mmol/L; FUJIFILM Pure Chemical Corporation, Osaka, Japan) and MSG (3 mmol/L; NACALTESQUE, INC., Kyoto, Japan) solutions were prepared by dissolving these reagents in DW. The GJ and

taste component solutions were prepared every experimental day. The GJ was stored at -28°C until further use for the analysis of pungency components.

Participants

The participants consisted of 19 Japanese students (8 males and 11 females) who were in their 20s. To avoid the effects of hunger on salivary secretion, the experiments were conducted between 14:00 and 16:30. Smoking, drinking, heavy exercise, and excessive intake of chili pepper, ginger, and coffee were prohibited from the day before to the day of the experiment to eliminate their effects on the autonomic nervous system. Participants underwent a washout period of >2 days between each experiment.

Salivary secretion measurement

Participants were asked to rinse their mouths, after which excess saliva was removed from their mouth. Subsequently, they were asked to hold 3 g of DW ($n = 19$), GJ ($n = 13$), CA ($n = 12$), and MSG ($n = 11$) solutions in their mouths for 1 min and then spit out the solution together with saliva. Participants were asked not to swallow their saliva or the test solutions while holding each sample. The expectorated saliva–solution mixture was weighed immediately, and the volume exceeding the initial 3 g of the taste compound solution was recorded as the amount of saliva secreted. Saliva was spat out at 1-min intervals for 8 min following oral stimulation and weighed immediately. The measurements were expressed in grams per minute or 8 min.

Autonomic nervous activity evaluation

Electrocardiography (ECG) was performed using a Polar H10 heart rate sensor (Polar Electro, Kempele, Finland) connected via Bluetooth to a tablet (Shenzhen Electron Technology Co., Ltd., Shenzhen, China). RR intervals were extracted from the ECG data collected continuously for 5 min immediately before and after each oral stimulation. The standard deviation of RR intervals (SDNN) was calculated to evaluate the overall autonomic nervous system activity, with lower SDNN values indicating sustained sympathetic nervous system dominance or reduced parasympathetic nervous system modulation²⁴). The root mean square of successive differences between RR intervals (RMSSD) was calculated to assess parasympathetic nervous system

activity. Lower RMSSD values reflected diminished beat-to-beat variability, which indicates reduced vagal modulation associated with stress conditions²⁴. In addition, RR interval, SDNN, and RMSSD were analyzed, excluding participants with poor ECG measurements and those for whom RR intervals could not be detected because of strong noise in the ECG. Therefore, the number of participants was 10–17 in these parameters. RR interval, SDNN, and RMSSD were expressed in milliseconds.

Pungency components in GJ determination

Pungency components (6-gingerol, 6-shogaol, and zingerone [Tokyo Chemical Industry Co., Ltd., Tokyo, Japan]) in GJ were detected by high-performance liquid chromatography (Shimadzu Co., Ltd., Kyoto, Japan), as described previously²⁵. An Inertsil ODS-3 column (4.6 mm i.d. × 150 mm, 5-μm particle size; GL Sciences Inc., Tokyo, Japan) was used in separating the components. GJ (150 μL) and methanol (450 μL) were mixed, vortexed, and sonicated for 30 min. Subsequently, the sample was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was collected and filtered through a 0.22-μm polytetrafluoroethylene filter (Starlab Scientific Co., Ltd., Shaanxi, China). The following analysis conditions were used: mobile phase A, acetonitrile; mobile phase B, ultrapure water; 60%–0% mobile phase B (0–12 min), 0%–60% mobile phase B (12–20 min); injection volume, 10 μL; flow rate, 1.0 mL/min; column oven temperature, 40°C; and UV/Vis detection wavelength, 230 nm. The results are expressed in μmol per L.

Statistical analysis

Values are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using Excel 2019 (Microsoft Japan Co., Ltd., Tokyo, Japan) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (version 4.0.3, The R Foundation for Statistical Computing, Vienna, Austria)²⁶. Differences between the solutions (DW, CA, MSG, and GJ) at each measurement timepoint were compared using the Tukey–Kramer test after performing one-way analysis of variance (ANOVA). The differences between the time courses of each solution were compared by one-way ANOVA and

Dunnett's test. A generalized linear mixed model (GLMM) was used to examine the main effects of the solutions and timepoints (before and after) on the RR interval, SDNN, and RMSSD. Because values for the RR interval, SDNN, and RMSSD were missing, a robust GLMM approach was selected. Each dependent variable was modeled using a gamma distribution with a log link function. Condition and time were set as fixed effects, and participants were designated as a random effect. Pairwise comparisons for multiple testing were performed using estimated marginal means, and *p*-values were adjusted using the Bonferroni method. The significance level was set at $\alpha = 0.05$.

Ethical considerations

This experiment was approved by the Human Ethics Committee of Graduate School of Human Development and Environment, Kobe University (Approval Number: 659-2) on December 15, 2023. Before the experiment, all participants were informed of the study objective and methods, measures to protect personal information, publication of research results, participants' right to withdraw consent, and voluntary nature of participation. Written informed consent was then obtained.

RESULTS AND DISCUSSION

Fig. 1 presents the changes in the salivary secretion amounts induced by GJ and taste component solutions. The amount of salivary secretions induced by GJ, CA, and MSG were the highest at the first 1 min and then decreased over time. Compared with DW, the amounts of salivary secretion significantly increased with GJ at 1–4 min and CA at 1 min ($p < 0.05$). Salivary secretions were the highest immediately after oral stimulation with GJ, CA, and MSG compared with other time points. This observation is consistent with the findings on CA, MSG, and capsaicin stimulation in previous studies^{15)–17)}.

Fig. 2 shows the total salivary secretions induced by GJ and taste component solutions. Compared with DW, the total salivary secretions induced by GJ ($p < 0.05$), CA, and MSG were significantly higher and that with GJ was the highest among the solutions used. Kan et al. found that intraperitoneal injection of ginger

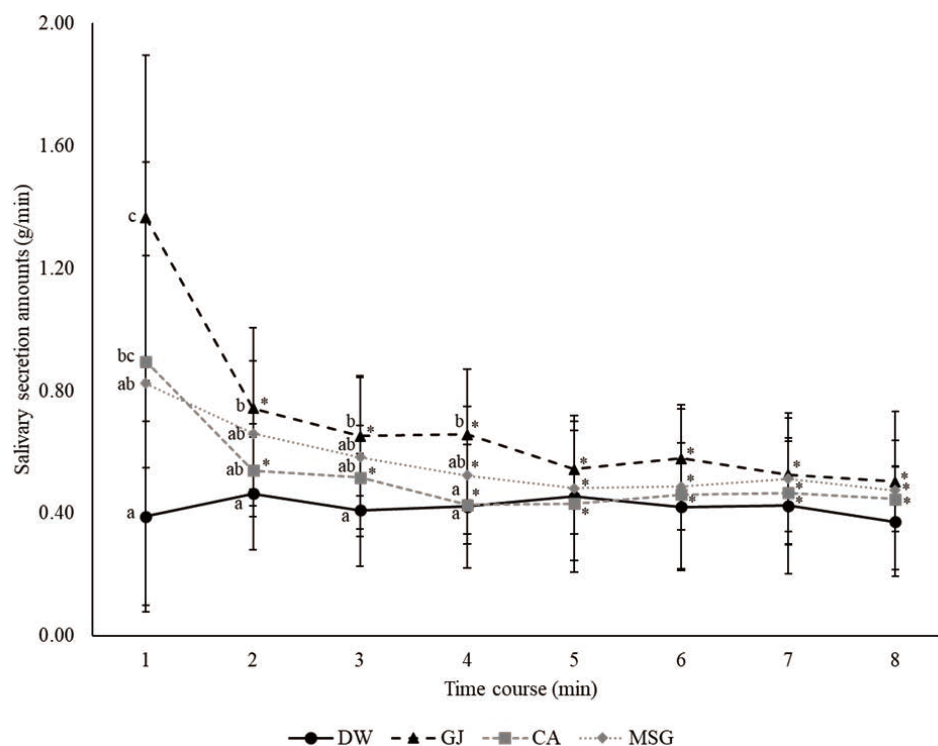


Fig. 1 Changes in the amounts of salivary secretion induced by oral stimulation with GJ and taste component solutions (n = 11–19)

DW, distilled water; GJ, ginger juice; CA, citric acid; MSG, monosodium L-glutamate. Values are shown as the means $\pm$ SD. ^{a–c}Values with different superscripts indicate a significant difference ($p < 0.05$, Tukey–Kramer test) compared between solutions at each time. * $p < 0.05$ (Dunnett's test), compared at 0 min vs. other time points for each solution.

extracts and 6-shogaol for 3 weeks increased salivary flow in mice ²¹). Patients who underwent radiotherapy and took methanolic ginger extract for 2 weeks had increased salivary secretions and improved xerostomia symptoms ²²). Furthermore, oral stimulation using ginger ethanol extract spray increased salivary secretion in patients with type II diabetes ²³). Although these studies demonstrate the ability of ginger extract to induce salivary secretion, the present study, to the best of our knowledge, showed for the first time that oral GJ intake, which can be implemented daily, stimulates salivary secretion in humans and may thus be useful for improving xerostomia symptoms and maintaining oral health. However, in this study, experiments that considered the number of molecules and the sensory intensity of CA, MSG, and pungency compounds were not performed. Therefore, further studies are needed to demonstrate whether GJ stimulates salivary secretion more than CA and MSG solutions.

Pre- and poststimulation RR interval, SDNN, and RMSSD values are shown in Fig. 3A–3C. The poststimulation RR intervals for all solutions were lower

than the prestimulation values ($p < 0.05$). No significant differences were observed in the pre- and

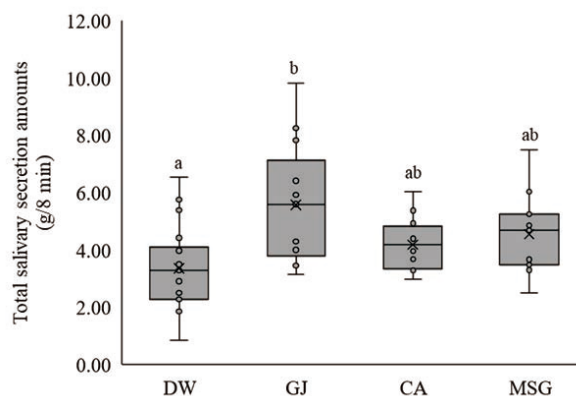


Fig. 2 Total amounts of salivary secretion by oral stimulation with GJ and taste component solutions (n = 11–19)

DW, distilled water; GJ, ginger juice; CA, citric acid; MSG, monosodium L-glutamate. The "x" mark indicates the mean. The box represents the median $\pm$ 25 percentile values, and the vertical bar indicates the minimum and maximum values. ^{a–b}Values with different superscripts indicate a significant difference ($p < 0.05$, Tukey–Kramer test), compared between solutions.

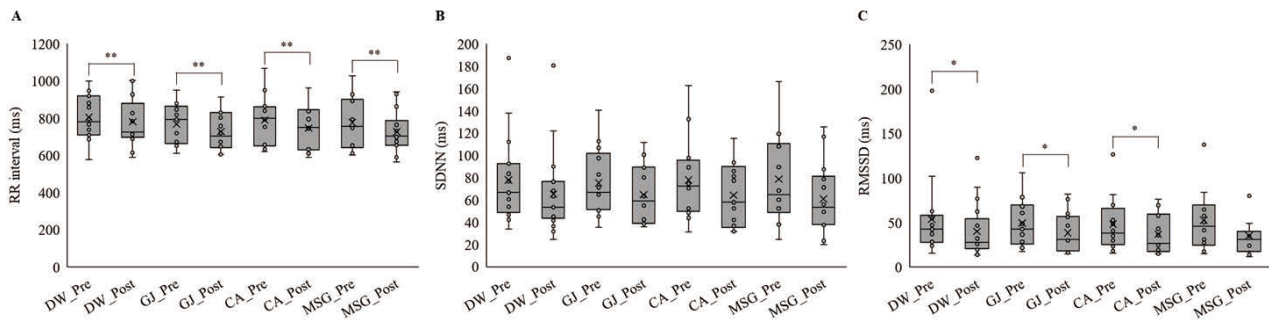


Fig. 3 Effect of oral stimulation with ginger juice on RR interval (A), SDNN (B), and RMSSD (C) ($n = 10-17$) DW, distilled water; GJ, ginger juice; CA, citric acid; MSG, monosodium L-glutamate. The “x” mark indicates the mean. The box represents the median $\pm$ 25 percentile values, and the vertical bar indicates the minimum and maximum values. * $p < 0.10$, ** $p < 0.05$ (generalized linear mixed model), compared between before and after stimulation with each solution.

poststimulation SDNN. Following oral stimulation with DW, CA, and GJ, the RMSSD values tended to be lower after than before stimulation ($p < 0.10$). No significant differences were observed in the RR interval, SDNN, and RMSSD between solutions. Oral stimulation with all solutions tended to increase sympathetic nervous activity; however, compared with the other solutions, GJ did not significantly affect autonomic nervous activity.

Taste stimulation of the oral cavity activates the salivary nuclei in the medulla oblongata, rapidly inducing salivary secretion via autonomic nervous activity in the facial and/or glossopharyngeal nerve¹². Furthermore, salivary secretion is promptly elicited by mechanical (e.g., chewing) and chemical (e.g., pungency) stimuli through the activation of the trigeminal nerve¹². For example, stimulation with CA (sourness), saccharose (sweetness), and capsaicin (pungency) induces immediate salivary secretion^{17,27}. In this study, oral stimulation with GJ, CA, and MSG may have induced immediate salivary secretion through these mechanisms. The decrease in poststimulation RR intervals and RMSSD indicated a switch to sympathetic nervous activation from autonomic nervous activity during prestimulation, but with slight differences. Furthermore, the SDNN of GJ did not vary significantly between pre- and poststimulation. Thus, oral stimulation with GJ did not strongly influence autonomic nervous activity.

However, previous studies have found that the pungency components of ginger extract activated the sympathetic nervous system^{7,8}, which contradicts the results of the present study. Although intraperitoneal injection of 6-paradol stimulated sympathetic nervous

activity in brown adipose tissue in rats²⁸, oral injection of 6-gingerol lowered this activity²⁹. Therefore, since the effects on autonomic nervous activity differ with the ginger pungency component used, oral stimulation with GJ may not affect autonomic nervous activity. The inconsistencies in findings on salivary secretion between the present study and previous studies should be investigated further. Furthermore, since oral stimulation with capsaicin induces sympathetic nerve activation¹⁷, induction of autonomic nervous activity during GJ stimulation should also be evaluated in future studies.

6-Gingerol and 6-shogaol were detected in GJ at concentrations of 177.50 ± 26.23 and 1.34 ± 0.25 $\mu\text{mol/L}$, respectively. However, zingerone was not detected. Therefore, 6-gingerol was identified as the major pungency component in GJ. The taste thresholds for pungency of 6-gingerol and 6-shogaol are 57.7 $\mu\text{mol/L}$ (17 ppm) and 25.3 $\mu\text{mol/L}$ (7 ppm), respectively¹. When this taste threshold is exceeded, as observed with capsaicin (the major pungency component in chili pepper), salivary secretion is induced¹⁷. Moreover, in this study, 84.6% of participants perceived GJ as “pungent”; thus, salivary secretion induced by oral stimulation with GJ may be attributed to pungency components, such as 6-gingerol, present at concentrations that exceeded the taste threshold for pungency.

CONCLUSION

To our knowledge, this study is the first to demonstrate that oral stimulation with GJ increases salivary secretion, which may be due to its pungency components, such as 6-gingerol and/or 6-shogaol content.

The findings indicate that oral stimulation with GJ may increase salivary secretion and enhance oral health.

CONFLICT OF 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.

APPENDICES

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ショウガ汁を用いた口腔刺激が唾液分泌に及ぼす影響

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要旨：

本研究では、ショウガ汁（GJ）を用いた口腔刺激が唾液分泌に及ぼす影響を検証した。蒸留水（DW）と比較して、GJを用いた口腔刺激により唾液分泌総量が増加した。GJ刺激によって誘発された唾液分泌量は、クエン酸（CA）およびL-グルタミン酸ナトリウム（MSG）刺激時よりも高値を示した。自律神経活動の指標（RR 間隔, standard deviation of RR intervals [SDNN], root mean square of successive differences between RR intervals [RMSSD]) は、試料（DW、GJ、CA、および MSG）間で差はみられなかった。GJ の辛味成分として、6-ジンゲロールと 6-ショウガオールが含まれ、主要な辛味成分は 6-ジンゲロールであった。従って、GJ 刺激による唾液分泌量の増加は、6-ジンゲロールや 6-ショウガオールなどの辛味成分によって誘発された可能性がある。以上より、GJ の口腔刺激が唾液分泌量を増加させ、口腔の健康維持に役立つ可能性が示唆された。

キーワード：ショウガ、唾液、辛味、6-ジンゲロール、心拍変動（HRV）

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