Mastication of Hard Gumi Decreases the Gustatory Threshold for Sodium Chloride

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Summary In recent years, there has been an increase in the number of hypertensive diseases and the various diseases associated with them. A major cause of these is excessive salt intake. The purpose of the present study was to examine whether chewing hard foods lowers the saltiness threshold. Fifteen subjects (fourteen women and one man) participated in the present study. Two types of gummies are available as ingredients: hard and soft gummies. The saltiness thresholds before and after chewing of each gumi were studied using 11 different NaCl solutions. Then, points of subjective equality (PSEs) were calculated to detect changes in the saltiness for each subject. In the soft Gumi condition, there was no significant difference in PSE for the saltiness between before and after ingesting Gumi (p>0.05), while in the hard Gumi condition, the PSE for the saltiness significantly decreased after ingesting Gumi compared with the value of before ingesting Gumi (p=0.001). From these results, we concluded that sensitivity to saltiness would increase after mastication of hard foods such as hard Gumi.

Key Words saltiness threshold, points of subjective equality, GLMM, taste, perception

In recent years, there has been an increase in the number of hypertensive diseases and the various diseases associated with them. A major cause of these is excessive salt intake (1). How can you prevent salt overdose? One of the causes of excessive salt intake from daily diet is an elevated salt taste threshold. A “threshold” is the lowest concentration of a stimulus at which a person can get close to the taste of that stimulus. As we age, the number of taste bud cells decreases, so we are less likely to perceive stimuli to saltiness, and the threshold increases, often resulting in excessive salt intake (2). Therefore, it can be said that increasing the sensitivity to saltiness and lowering the “saltiness threshold” have the effect of preventing “excessive salt intake”. Various studies have focused on the relationship between taste sensitivity and food preference, food behavior, and chewing and swallowing (3–6). For example, it is well known that the amount of saliva secreted is associated with the amount of chewing (7). In addition, Matsuo et al. reported that saliva secretion changes the sensitivity to four tastes (salt, sour, sweet, and bitter), and that the sensitivity to taste is reduced when saliva is low (8). This was particularly evident in the sensitivity to salt (8–10). Furthermore, there is a strong relationship between chewing strength and saliva secretion (11). Therefore, it is possible that chewing hard foods may stimulate saliva secretion and increase salt sensitivity compared to chewing soft foods. A previous study found that masticatory muscle group activity, as measured by electromyography (EMG), was higher when chewing Gumi than when chewing gum (12). In this study, the increase in mastication during chewing Gumi may significantly affect on saliva secretion and perception of saltiness more than during chewing gum (12). In the study, we tested the hypothesis that chewing hard foods may increase saliva secretion and increase sensitivity to taste and decrease saltiness threshold more than chewing soft foods by using hard and soft Gumi that can be easily adjusted for composition and hardness.

Methods Participants. Fifteen young, healthy subjects (14 female, 1 male; age: 19.5±0.5 y, mean±SD) participated in the present study. The number of subjects was calculated using G*Power (effect size=0.25, alpha error probability=0.05, power (1−β error probability)=0.95) (13) to calculate the number of subjects required before the study. The subjects did not have any neurological or endocrine disorders. In addition, all the subjects were non-smokers and did not use any drugs. The study protocol was approved by the Ethics Committee of the Niigata University of Health and Welfare (No.
Table 1. The component of Gumi (hard Gumi and soft Gumi).

| Component                          | Hard (%) | Soft (%) |
|------------------------------------|----------|----------|
| Starch syrup                       | 55.083   | 58.721   |
| Sugar                              | 28.376   | 30.251   |
| Gelatin                            | 10.907   | 5.453    |
| Grape juice                        | 2.380    | 2.38     |
| Vegetable fats and oils            | 0.146    | 0.146    |
| Starch                             | 0.700    | 0.700    |
| Acidulant                          | 1.876    | 1.876    |
| Fragrance                          | 0.319    | 0.275    |
| Coloring agents (anthocyanin and gardenia pigment) | 0.178 | 0.178 |
| Brightener                         | 0.005    | 0.005    |
| Carry-over                         | 0.030    | 0.015    |

17737-161007). Each subject participated by obtaining written informed consent after being briefed by the experimenter on the aims and methods of the study.

Procedure. For all subjects, the saltiness threshold was tested in two conditions (hard Gumi chewing and soft Gumi chewing). The subjects did not consume any food or drink other than water for at least 2 h before the study. We also avoided salty foods and caffeinated drinks (coffee, tea, green tea, etc.) on the day of the test and the day before. The ingredients of the Gumi that used in the present study are shown in Table 1. Hard and soft Gumi differed in gelatin content and aroma and other tastes (sweet, sour, and bitter) do not affect the perception of saltiness (14, 15). In addition, the components of the Gumi that directly affect these tastes (sugar, grape concentrates, fruit juices, and acidifiers) are similar in both the hard and soft Gumi groups, so they are unlikely to affect the saltiness threshold.

The saltiness thresholds before and after chewing of hard and soft Gumi were tested using 11 different NaCl solutions of 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.1, and 0.12% (weight/volume) (16, 17). The test procedures and the solute concentrations used in the present study were in accordance with the previous studies (16, 17). The salty solution was placed in a container at room temperature.

For testing for saltiness threshold, the solution was dripped onto the tongue of each subject using a 1.0 mL syringe after wearing an eye mask and headphones. The subjects were instructed to hold the fluid solution in their mouths for 5 s and then spit it out. After each tasting, the subjects were asked to remove their eye mask and point to the NaCl solution he had supposedly tasted. The subjects followed the same protocol and they were instructed to taste the solution and rinse their mouths with water once between solutions. The threshold test for each subject was always started from 0% NaCl solution as described in the previous studies (16, 17), which is a stimulating concentration that can secure a sufficient number of steps on both sides of the starting point to determine the exact threshold. The average response for each solution is calculated for each subject. Since the duration of saliva secretion by mouth rinsing to food intake is more than 10 min (18), so the decrease in oral saliva due to mouth rising may be due to a salty taste. It did not affect the consideration of the threshold.

Results
The hardness of Gumi
The hardness of hard and soft Gumi was 24.43 ± 13.12 (10⁶ Pa) and 6.03 ± 1.00 (10⁶ Pa). The hardness of hard Gumi was significantly harder than the hardness of soft Gumi ($p<0.0001$).

Effect of Gumi hardness on the salty taste threshold
We analyzed the saltiness threshold data using GLMM

Data analysis. We calculated point of subjective equality (PSE) to identify the threshold for each subject as described in the previous study (19). For the detection of PSE, the relationship between each salt concentration and the probability of searching for saltiness was fitted using a logistic curve for each subject. We calculated PSE that subjects did or did not feel taste. To manage nested data and to avoid type I and II errors, we used generalized linear mixed model (GLMM). The GLMM analysis was performed based on a unique selective coding model with fixed effects of “hardness of Gumi” (hard and soft) and “time” (before and after). In this model, “subject” (subject ID) is added as a random effect in consideration of the difference in reaction tendency between subjects and the difference in sensitivity to fixed effects. Thus, the intercept and slope of each fixed effect include the random effect of the “subject,” suggesting that this “maximum random effect structure” is the best way to perform a verifiable hypothesis test. After the GLMM was calculated, ANOVA was performed using the data obtained from the GLMM. The Kenward-Roger approximation of degrees of freedom was used for this analysis. For multiple comparisons, the difference between the least-squares means and confidence intervals of fixed factors in the linear mixed-effects model was calculated. $p$-value is obtained by a likelihood ratio test of the full model with the problem effect and the model without the problem effect. We used the function of lmer of the lmerTest package available in R (Version 3.2.4) (20). $p$-values less than 0.05 were considered statistically significant.
Hard Gumi Increases Sensitivity to Saltiness

It has been reported that harder substances require greater chewing power (11), and Matsuo et al. (8) suggested that salinity affects the saltiness threshold. Matsuo et al. (8) also reported that decreased salivary secretion reduced sensitivity to four tastes (saltiness, sourness and acidity, sweetness, and bitterness). In addition, they reported that the effect of saliva secretion was most pronounced in the sensitivity to salt (8). These results are consistent with the results of the present study, chewing hard Gumi might lead chewing power and promote saliva secretion, it is likely that these reduced the saltiness threshold. In addition, Anderson et al. (11) showed that saliva secretion increases with increased masticatory power in rats and rabbits. These findings may not necessarily be fully transferable to humans, since all of these studies were conducted in animals (11). In a human study, Hasegawa et al. (21) showed the gum mastication increased the saliva secretion. In the present study, we investigated the effect of substance hardness on the saltiness threshold, then showed that chewing hard Gumi reduced the saltiness threshold.

The taste threshold varies depending on various factors such as stimulation method, stimulation position, composition of the test solution, and temperature (22). Various substances are known to affect the taste threshold, such as sodium, HCO\textsubscript{3}\textsuperscript{-}, and NaHCO\textsubscript{3} (23). However, in the present study, these variables have a uniformity between hard and soft Gumi conditions. Therefore, we do not consider that the factors listed above have any effect in the present study.

In the present study, the saltiness threshold was found to be lower with the chewing hard Gumi. Eating hard foods before meals or including hard foods in the meal creates the action of chewing hard foods, and by lowering the saltiness threshold, the amount of salt intake would be reduced, which may lead to the prevention of hypertension. Finally, the effects of chewing hard Gumi should be examined in the context of other gustatory thresholds (salt, sour, sweet, and bitter), and saltiness thresholds should be examined using different stimuli, such as electrical stimulation with an electric gustometer. Since previous study (24) have reported an increase in saliva volume as a function of hardness, it is possible that a similar increase may be observed with harder Gumi than those used in the present study, but further studies are needed. In addition, the accuracy of the present study would be improved by unifying the conditions of the subjects at the time of Gumi consumption (number of chews, exercise restrictions before and after the study, flavors etc.). Although this experiment was conducted with young people, it is necessary to conduct a study with the elderly because it is necessary to investigate whether the same thing can be said for the elderly. In addition, since this was a cross-sectional study, a longitudinal study would enhance the accuracy of this study.

**Discussion**

The precious result of the present study was that the saltiness threshold decreased after hard Gumi ingestion compared with soft Gumi ingestion and before the Gumi ingestion. The results of the present study indicate that sensitivity to salt may be increased by mastication of hard foods. Examination for salt taste threshold and other protocols are in accordance with previous studies and are reasonable. To our knowledge, this is the first report that salty taste threshold had changed by ingesting foods. The present study showed that chewing hard Gumi significantly reduced the salt taste threshold as shown in Fig. 1 which suggesting that chewing hard substances increases the sensitivity to salt.

It has been reported that harder substances require and calculated the results of repeated measures ANOVA. Significant differences in saltiness thresholds were found to be due to time (between pre- and post-Gumi ingestion) (density of degree of freedom=45.9, \( F = 7.768, p=6.61\times10^{-10} \)), hardness of Gumi (density of degree of freedom=41.59, \( F = 5.328, p=3.74\times10^{-6} \)), and the interaction between time and Gumi types (hard versus soft Gumi) (density of degree of freedom=20.99, \( F = -4.82, p=9.14\times10^{-5} \)). According to the simple main effects test, significant differences in saltiness thresholds were found between pre- and post-test (degree of freedom=42.00, \( t \)-value=\(-2.96, \text{ lower CI} = -0.008, \text{ upper CI} = -0.002, p=0.005 \)), and between pre and post-test in hard Gumi (degree of freedom=42.00, \( t \)-value=\(-5.33, \text{ lower CI} = -0.018, \text{ upper CI} = -0.008, p=2.00\times10^{-16} \)). In the hard Gumi condition, the salty taste threshold before Gumi ingestion was 0.047±0.022 g/mL and after Gumi ingestion was 0.034±0.017 g/mL. In the soft Gumi condition, the salty taste threshold before Gumi ingestion was 0.043±0.021 g/mL and after Gumi ingestion was 0.045±0.022 g/mL. Figure 1 shows the salty threshold for hard and soft Gumi before and after ingestion of the Gumi.

**Conclusion**

The results of the present study indicate that sensitivity to salt may be increased by mastication of hard...
foods. Examination for salt taste threshold and other protocols are in accordance with previous studies and are reasonable. To our knowledge, this is the first report that salty taste threshold had changed by ingesting foods.

**Authorship**

Research conception and design: KSu and KSh; experiments: KSu and KSh; statistical analysis of the data: RS, MM, NH, CH, AG, NS, and HI; interpretation of the data: RS, MM, NH, CH, AG, NS, KSu, and KSh; writing of the manuscript: RS, MM, NH, CH, AG, NS, KSu, HI, and KSh.

**Disclosure of state of COI**

No conflicts of interest to be declared.

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