ORIGINAL

Dietary supplementation with monosodium glutamate with dietary balance such as protein, salt and sugar intake with increasing T1R3 taste receptor gene expression in healthy females

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Abstract : We previously showed that chemotherapy-induced dysgeusia was associated with lingual taste receptor gene expression, and monosodium glutamate (MSG) improved dysgeusia by upregulating taste 1 receptor 3 (T1R3) gene expression. In recent years, decreased taste sensitivity has also been reported in some young people, and these are partly due to their disordered eating habits. From these background, we investigated the effects of MSG supplementation on taste receptor expression and dietary intake in healthy females. Fifteen young healthy volunteers were enrolled for the present crossover study and divided in two groups (dietary supplementation with MSG at 2.7 g/day or 0.27 g/day). The relative expression of T1R3, a subunit of both umami and sweet taste receptors, in the tongue was assessed by quantitative PCR analysis. Food intake was assessed by food frequency questionnaire (FFQg), and body composition was measured using Omron HBF-701. T1R3 expression levels in the tongue and taste sensitivity increased significantly in participants who consumed <10 g of MSG daily, whereas no alteration was observed in participants who consumed >10 g of MSG daily. Furthermore, protein, fat, and carbohydrate (PFC) balance and salt and sugar intake improved by MSG supplementation. In conclusion, MSG supplementation increased T1R3 expression in the tongue and improved dietary balance. J. Med. Invest. 68 : 315-320, August, 2021

Keywords **:** *monosodium glutamate (MSG), taste 1 receptor 3 (T1R3), dysgeusia*

INTRODUCTION

Dysgeusia is serious because it lowers quality of life and exacerbates pathological conditions, including weight loss and malnutrition. Taste sensitivity is an important factor for maintaining life as well as for enjoying diet (1). In addition to medication and aging, there are several causes of decreased taste sensitivity. Cancer therapy, diabetes, infection and inflammation, vitamin B12 or zinc deficiencies, or dry months are well-known causes. In addition, COVID-19 induces chemosensory loss, such as loss of taste and smell. Many cases of dysgeusia caused by COVID-19 have been reported worldwide (2-6).

Sense of taste is critical for the identification of healthy nutrients and avoiding unhealthy compounds, such as spoiled or poisonous food. However, even in young people, taste sensitivity is sometimes lowered, without the being person aware of it (7). In general, reduced taste sense leads to inadequate dietary intake, resulting in malnutrition or overeating, whereas low levels of taste sensation are also caused by food habits in healthy subjects (8).

The basic taste modalities are sweet, sour, bitter, salty, and umami, which are perceived by specific taste receptors in the taste buds on the tongue (9). Umami taste was identified more than 100 years ago by a Japanese scientist, Dr. Kikunae Ikeda ; however, it has been recognized as a fifth distinct taste owing to the identification of its receptors in the last two decades (10-12). Umami and sweet taste receptors are heterodimeric proteins composed of taste 1 receptor 1 (T1R1) and T1R3 and T1R2 and T1R3 subunits, respectively (13), whereas bitter taste receptors are monomers of the 25 types of T2R subunits (14). Previously, we reported that lingual taste receptor gene expression was associated with chemotherapy-induced dysgeusia in patients with head and neck cancer (15). T1R3 gene expression decreases after chemotherapy, whereas T2R5 gene expression increases, which is correlated with decreased taste sense.

Monosodium salt of L-glutamate (MSG) is an umami compound used worldwide as a seasoning and is detected by umami taste receptors in the tongue (16). We have recently found that stimulation of taste buds with MSG results in upregulation of T1R1 and T1R3 expression levels in the tongue of patients with head and neck cancer undergoing chemotherapy. Here, we analyzed the effect of MSG supplementation in healthy young females. MSG was added to rice at each meal three times per day for seven days. A mucosal scraping sample was obtained from the foliate papillae on the tongue of study subjects to assess T1R3 abundance by real-time PCR analysis. In addition, we examined the effects of dietary supplementation with MSG on daily dietary intake.

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SUBJECTS AND METHODS

Subjects and study design

The study was approved by the Tokushima University Hospital Committee for Medical Ethics (approval number : 2405), and written informed consent was obtained from each patient prior to enrollment. The Study design complied with the guidelines of Declaration of Helsinki, and all methods were performed in accordance with the relevant guidelines and regulations of the Institutional Ethics Committee of Tokushima University Hospital, Japan. The study was designed as a crossover study. Fifteen healthy young participants were enrolled in the study. The participants were randomly divided into two groups : one group included eight participants who were supplemented with 2.7 g/day (0.9 g/meal) MSG for the first week. After 2 weeks of wash out, they were then supplemented with 0.27 g/day (0.09 g/meal) MSG for a week. Another group included other seven participants who were supplemented with 0.27 g/day MSG for the first week and 2.7 g/day for another week. MSG was provided in the form of seasoning, with a double-blind addition of a seasoning containing 0.9 g or 0.09 g of MSG.

Dietary MSG supplementation

The patients were randomly divided into two groups. One group (group A) comprised seven participants (all seven were women) and another group (group B) comprised eight participants (of which seven were women). MSG powder was added directly to rice as a seasoning provided by Marumiya Co. Ltd. (Tokyo, Japan) (Table 1), and the powder was sprinkled over rice. The powder contained 0.09 g or 0.9 g of MSG for consumption three times per day for seven days.

Table 1. Nutritional composition of seasoning (0.9 g MSG)

Nutrients	amount
energy	13 kcal
protein	1.3 g
fat	0.4 g
carbohydrate	1.0 g
salt equivalent	0.5 g
sodium	200 mg
water	0.5 g
ash	0.5 g
glutamate	843.6 mg

RT-PCR analysis

The surface of the foliate papillae on the tongue of patients was scraped with a small spatula after local anesthesia with 4% lidocaine to collect a sample of the lingual mucosa (24). Scraping was performed immediately before and one week after the first and second doses of chemotherapy. All tissue scrapings were immediately mixed with RNAlater solution (Ambion, Austin, TX, USA), and RNA was extracted using an RNAqueous phenol-free RNA isolation kit (Ambion) and amplified using the CellAmp Whole Transcriptome Amplification Kit Version 2 (Takara Bio, Shiga, Japan). Total RNA (1 μg) was reverse-transcribed in a final volume of 20 μL using the Primescript RT Reagent Kit (Takara Bio). The resulting cDNA (50 ng) was subjected to real-time PCR using specific primers in a final volume of 10 μL using StepOnePlus Real-Time PCR System (Life Technologies, Waltham, MA, USA). The sequences of the primer sets used (forward and reverse, respectively) were 5′-TTCCCCCAGTACGTGAAGAC-3′ and 5′-CAGAGAACGTCTGGTGGTGA-3′ for human T1R3 and 5′-GAAATCCCATCACCATCTTCCAGG-3′ and 5′-GAGCCCCAGCCTTCTCCATG-3′ for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Invitrogen, Waltham, MA, USA). PCR products were quantified by fit-point analysis, and the expression of T1R3 was normalized to that of GAPDH.

Daily dietary intake

Dietary intake was calculated by a registered dietician by assessing food frequency questionnaire (FFQg) version 4.0. Average energy and all nutrients intake over seven days was compared between the weeks before and after the first and second doses of supplementation. To assess glutamate intake, we also assessment dietary intake by 24-hour diet recall assessment and calculated amount of glutamate using Japan Food Standard Component Table 2015, An Amino Acid Component.

Body composition

Body weight and body composition were assessed using a TANITA dual-frequency body composition analyzer (TANITA 320).

Statistical analysis

Data are presented as mean ± standard deviation. Paired t-test was performed after assessing the normality of data. Multiple comparisons were performed using the Kruskal–Wallis test and two-way analysis of variance. If an overall significant difference was detected, the Tukey–Kramer test was applied to identify pairs showing significant differences. All statistical analyses and graph generation were conducted using JMP (SAS Institute, Tokyo, Japan) or PRISM 7 software (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

RESULTS

Participant characteristics

A total of 15 healthy participants [21–26-years-old (23.2 ± 1.6 years)] with no medication were enrolled in the study (Table 2). There were no differences in body composition and energy intake between the groups before supplementation. The abundance of T1R3 varied from individual to individual and was not associated with nutritional intake, such as sugar and glutamic acid, and dietary intake, such as sugar, confectionery, and favorite beverages (data not shown).

Change in food intake and body composition and T1R3 expression after MSG supplementation

We compared energy and food intake before and after supplementation with MSG. Intake of 0.27 g/day MSG did not change food intake, including energy and salt intake, whereas after 2.7 g/day MSG supplementation, energy, salt and sugar intake reduced (Table3, Figure1A-C). Intake of snacks were decreased after 2.7 g MSG intervention, suggesting that intervention reduced these nutrients such as salt and sugar. Other nutrients such as protein and fat were not altered (Table3). Body composition, i.e., body fat, lean body fat, and intracellular and extracellular body fat did not change throughout the study (data not shown). In addition, nausea, vomiting, and diarrhea did not occur after supplementation.

Next, we analyzed lingual T1R3 gene expression before and after MSG supplementation. Supplementation with 0.27 g/day

Table 2. Characteristic of participants

	Group A (n = 8)	Group B (n = 7)	*p*
Demographics			
Age (year)	23.8 ± 1.5	22.9 ± 1.6	p = 0.3424
Gender (male/female)	0/8	0/7	
Anthropometrics			
weight (kg)	53.7 ± 6.4	54.1 ± 7.3	p = 0.2261
BMI (kg/m2)	21.4 ± 3.1	21.8 ± 2.8	p = 0.1457
body fat (%)	29.7 ± 7.6	28.9 ± 7.7	p = 0.3164
lean body mass (kg)	36.5 ± 5.9	37.5 ± 9.2	p = 0.3919
Blood pressures (BP, mmHg)			
Systolic BP	128.5 ± 10.5	124.5 ± 17.9	p = 0.3862
Diastolic BP	78.5 ± 12.8	80.5 ± 16.9	p = 0.2661
Hypertension (> 140/90 mmHg)	0	0	n.s
Herat Rate	70.2 ± 10.6	71.3 ± 12.2	p = 0.1125
Dietary assessment			
Energy intake (kcal/day)	2064.0± ± 239.4	1917.5 ± 251.5	p = 0.2612
protein intake (g/day)	58.0 ± 6.9	60.8 ± 5.1	p = 0.3428
carbohydrate intake (g/day)	224.0 ± 56.5	265.5 ± 48.9	p = 0.2827
sugar intake (g/day)	9.5 ± 4.9	8.9 ± 3.7	p = 0.2812
salt intake (g/day)	8.5 ± 3.9	18.2 ± 4.5	p = 0.6525

Characteristics of participants before the intervention.

Table 3. dietary intake before and after MSG investigation

	Pretreatment	After 0.27 g MSG	After 2.7 g MSG
Energy intake (kcal/day)	2032.0 ± 246.5	2017.4± ± 257.8	1872.4 ± 210.8*
protein intake (g/day)	59.9 ± 6.3	61.8 ± 4.1	60.8 ± 4.1
carbohydrate intake (g/day)	223.0 ± 54.7	245.5 ± 48.9	245.5 ± 40.1
Fat intake (g/day)	45.6 ± 14.2	43.0 ± 11.2	41.0 ± 11.7
sugar intake (g/day)	10.5 ± 4.9	10.1 ± 3.4	8.6 ± 3.1*
salt intake (g/day)	11.9 ± 3.9	10.2± ± 4.5	8.1 ± 1.8*
Glutamate (g/day)	15.4 ± 3.9	16.7 ± 3.1	16.3 ± 4.1

*p < 0.05 versus pretreatment

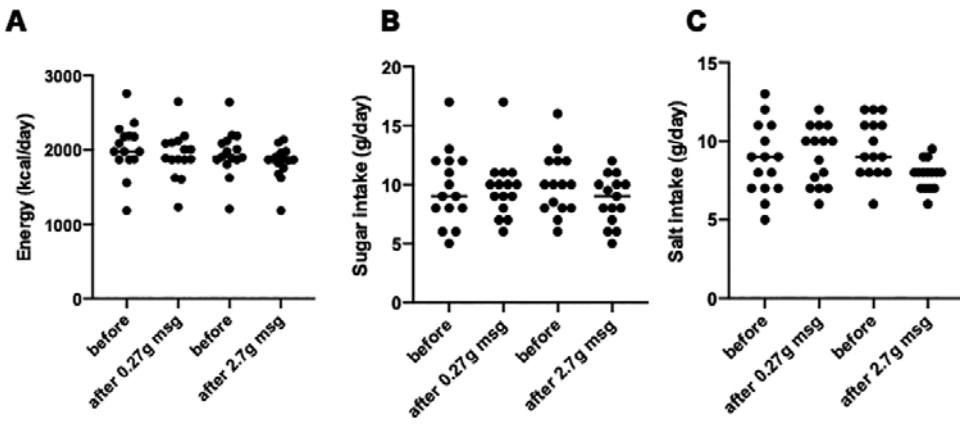

Figure 1. Change of energy, salt and sugar intake during intervention.
A) Energy intake, B) Salt intake, C) Sugar intake, were investigated before and after 0.27 g/day MSG intervention and before and after 0.27 g/day MSG intervention. One-way ANOVA and paired t test were performed. N = 15, each.

MSG did not change T1R3 gene expression (Figure2A). After 2.7 g/day MSG supplementation, eight participants (53%) showed increased lingual T1R3 gene expression, but it remained unchanged in other participants (Figure2B-C).

Stratified analysis of T1R3 gene expression after MSG supplementation

Therefore, further analysis was performed to clarify why some participants exhibited increased T1R3 gene expression but others did not. Comparing the group in which T1R3 gene expression increased by supplementation of 2.7 g of MSG and the group in which there was no change, there was no significant difference in energy intake, but the group with increased T1R3 gene expression had lower protein intake (43.2 g/day vs. 67.6 g/day ; $p < 0.05$), and the average intake of glutamate was also less than 10 g per day before intervention (9.6 g/day vs. 21.4 g/day) (Figure3A-B). In addition, the group with increased T1R3 expression had higher salt and sugar intake before intervention (Figure3C-D) before intervention. In the group with increased T1R3 gene expression, such low protein intake and high salt and sugar intake improved after MSG addition. That is, protein

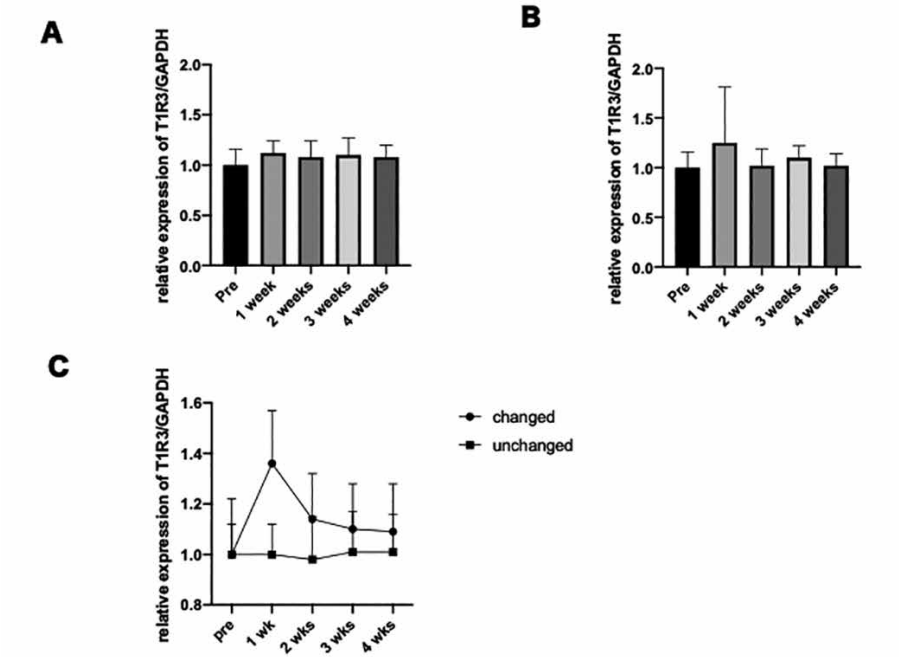

Figure 2. Lingual T1R3 gene expression during intervention.
A) Lingual T1R3 gene expression before and after 1, 2, 3 and 4 weeks of supplementation with 0.27 g/day MSG intervention. B) Lingual T1R3 gene expression before and after 1, 2, 3 and 4 weeks of supplementation with 2.7 g/day MSG intervention. N = 15, each. C) Comparison of Lingual T1R3 gene expression between T1R3 changed and unchanged groups. One-way ANOVA were performed. Changed ; n = 8, unchanged ; n = 7

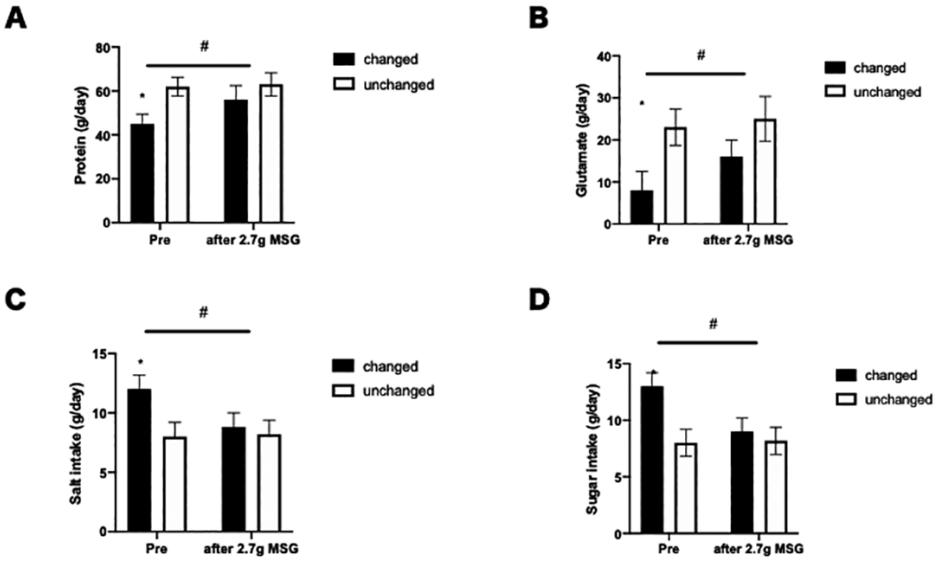

Figure 3. Comparison of protein, glutamate, salt and sugar intake amount.
A) Protein, B) Glutamate, C) Salt, D) Sugar intake amounts were showed in two group. Changed : group that T1R3 gene expression was increased by 2.7 g/day MSG supplementation ; closed bar (n = 8). Unchanged : group that T1R3 gene expression was unchanged after 2.7 g/day MSG supplementation ; opened bar (n = 7). *$p<0.05$, unpaired student t-test.

intake increased after the intervention of 2.7 g MSG, salt and sugar intake decreased, and was within the generally recommended dietary intake range. There was no clear difference in energy intake between the group with increased T1R3 gene and the group without change in T1R3 gene after MSG intervention. This suggests that MSG intake changed the dietary habits or dietary components.

DISCUSSION

MSG is a sodium salt of glutamic acid (glutamate, Glu). Glutamate is a non-essential amino acid, and is the most abundant amino acid found in nature (17). However, MSG is a controversial compound regarding its use in the food industry. In this study, we investigated the effect of supplementation with MSG on dietary balance and taste receptor T1R3 gene expression in healthy females. We focused on the effects of MSG and not on those of other umami compounds, such as 5′-ribonucleotides guanosine monophosphate and inosine monophosphate, as our preliminary work showed that MSG was the only compound that increased T1R3 expression in the tongue of mice (unpublished data). The human T1R1/T1R3 heterodimer is activated by glutamate (18). We previously observed that during the first round of chemotherapy (cisplatin only or a combination of cisplatin and 5-fluorouracil) during chemoradiotherapy resulted in a decrease in T1R3 expression levels in the lingual mucosa and taste sensitivity in patients with advanced head and neck cancer. These effects were attenuated by dietary supplementation with MSG during the second round of chemotherapy (unpublished data). In addition, we focused on T1R3 because chemotherapy (but not radiation therapy) reportedly reduces T1R3 expression in the tongue of patients with head and neck cancer without altering T1R1 or T1R2 expression (15).

MSG is recognized as the main ingredient of umami taste. Recently, the health benefits of umami has received considerable attention. The use of umami can help reduce excessive salt intake, which contributes to cardiovascular disease. Differences in the salt-exposed environment at birth and preference for salty taste may affect the sense of taste (19). In addition, Mizuta *et al*. demonstrated that umami taste disorder is a predictor of obesity and suggested that umami contributes to meal satisfaction (20). Supplementation of MSG in elderly patients promotes gastric acid and bile acid secretion and contributes to increased dietary intake and improved nutrient absorption rate, resulting in improved nutritional status (21, 22). Oral administration of MSG also increased gastrointestinal mRNA and protein levels of T1R1 and T1R3 in piglets (23). Although the precise mechanisms of these effects remain unclear, it is likely that the interaction of MSG with T1R1/T1R3 umami receptors in the tongue of patients with head and neck cancer activates intracellular signals that elicit the expression of T1R3, thereby compensating for the inhibitory effect of chemotherapy.

In this study, we aimed to determine whether the increase in T1R3 gene expression by MSG supplementation may be dependent on glutamate intake from the original diet. In subjects with low glutamate intake and low protein intake, the state of "glutamate undernutrition" suppressed T1R3 gene expression, which was considered to be improved by adding 2.7 g of glutamate daily. In these subjects, in addition to low protein intake, high salt and sugar intake was corrected by supplementation with MSG. There is a strong association between sweet and umami tastes, and supplementation with MSG may support reduced sugar intake (24). Although MSG includes sodium, taste satisfaction with MSG may also have contributed to reduced salt intake.

The present study has several limitations. First, taste sensation was not assessed using an accurate taste test. Only interviews were conducted regarding dysgeusia, and it was confirmed that none of the subjects had any subjective abnormalities. However, a more accurate taste test, such as the whole-mouth gustatory test, is required to be performed because sometimes even young healthy subjects present dysgeusia. Second, the number of subjects was relatively small and the study was designed as a crossover study. Third, only women were included this study. Gender differences are also considered to be involved in taste and gene expression, but we have not been able to examine gender differences in this study. Thus, further randomized clinical trials are required to confirm our findings.

In conclusion, this study showed that dietary supplementation with MSG improves dietary balance by increasing T1R3 taste receptor gene expression in healthy females with an unbalanced diet.

CONFLICT OF INTEREST

The authors have no financial conflicts of interest to disclose.

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