Relationship between Measurement Salt Taste Threshold and Salt Intake in Japanese Primary School Students, Parents and High School Students Using Test Papers of Low Concentration Taste Threshold

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Abstract. Introduction: Because of the inspection of salty taste has a background developed mainly for extraction of taste disorders and the guidance on diet for patients, the information in healthy young people isn't detail. Recently, it reported that salt taste thresholds in university students using low concentration threshold test paper was about 60% as concentration as previously reports using taste paper.We used test papers of low concentration taste threshold to investigate the salt taste threshold in school children, their parents, and in high school students. Methods: The subjects comprised 26 families (26 fathers, 26 mothers, and their 26 children) and 25 female high school students. Salt intake per day was determined by measuring urinary salt excretion. Different concentrations of salt taste test papers (Salsave[®]) were used to evaluate the salt taste threshold. Results: Mean salt intakes (g/day) were 7.32±1.63 (children), 7.83±2.30 (mothers), and 9.05±2.73 (fathers). Mean salt taste thresholds (mg/cm²) were 0.28±0.33 (children), 0.33±0.25 (mothers), and 0.25±0.14 (fathers). High school students' mean salt intake was 8.52±3.00, and salt taste threshold was 0.28±0.13. Those salt taste threshold concentration were accuracy lower than that of previously reports. Also, significant positive correlations between recognized salt taste thresholds and salt intake were found in the children, boy's fathers and high school students (P < 0.05). Conclusion: Using low concentration test papers which not for sale because of unprofitable previously, could analyze the salty taste threshold of healthy volunteers in detail. The results suggested a relationship between daily salt intake and the salt taste threshold.

Key words: salt taste threshold, salt intake, salt taste check papers, young, student

Introduction

Reducing dietary salt intake is an important public health strategy for the prevention and treatment of hypertension and cardiovascular disease (1, 2). Traditional Japanese meals provide a large intake of sodium chloride (3). Recently, lifestyle diseases have increased with a background of excess intake of animal oils and fats and too little exercise, and they are becoming a social problem (4, 5). Also, convenience stores and home-meal replacements have made it possible to ingest meals easily and inexpensively without cooking in Japan (6). Furthermore, dining together as a family has decreased because of the workload of the parents and school attendance of the children. Thus, eating out and eating alone have increased in children (6, 7).

Home-meal replacements and meals eaten outside the home have a generally high sodium concentration and exceeding of meal intake standards is also occurring (7). Hypertension is generally considered to be one of the risk factors for developing cardiovascular diseases (1, 2, 8) through the consumption of highly flavorful food, especially foods with a high salt concentration (9). An increase in plasma sodium concentration due to excessive salt intake can increase the circulating plasma volume, increase blood pressure due to hypertonic pressure, and increase the amount of water drunk due to depression of the taste center (10). In addition, it is thought that appetite is promoted by a high-salt diet and tends to result in overeating (11). Therefore, it is expected that reducing both the salty taste of foods and salt intake will lead to the suppression of obesity related to hypertension and overeating.

Salty taste is one of the basic tastes in humans. It is speculated that salt intake habits of many years and the accumulation of experience and taste are related, and it is estimated that salt intake and the salt taste sensation are involved in comparisons between people with diseases such as hypertension and healthy people. Although studies have been performed to investigate this (12–14), this speculation has not been sufficiently verified in the dietary habits of healthy subjects (15, 16).

We thought that one reason of information on salty taste in healthy young people is limited is related to the background that taste test paper was sold for the main purpose of extraction of taste disorder and food instruction to patients. The taste test methods were developed from 1980 to 1995 that can be inspected to a wide range of concentration thresholds including low concentration thresholds. However the paper of low concentration range was not sale because of rarely demand of salt taste test about patients. Recently, Nishimoto et al reported the taste threshold value of university students was 60% of the previous report using low-density test paper of salt taste inspection paper manufactured by Toyo Roshi Co., Ltd., Futhermore, the concentration of the taste discs@ (Sanwa Kagaku Kenkyusho Co. Ltd.) which major used of taste test in Japan are 0%Nacl, 15 mg (0.3% Nacl), 62.5 mg (1.25% Nacl), 250 mg (0.7% Nacl), and so on. Also, the concentration of salt taste test papers (Salsave®; ADVANTEC Toyo Roshi Kaisha, Ltd.) are 0%Nacl, 0.6 mg/ cm 2 (0.9% Nacl), 0.8 mg/ cm 2 (1.1% Nacl), and so on. Also, even if the existing literature is examined, studies in which the measurement of the concentration of 0.4% Nacl or less has been carried out in detail cannot be found out of our knowledge. Measure of the salt taste threshold of both children, children's parents and young people with salt paper is lead to knowing the current state of salt taste of healthy subjects and changes with aging. Also, it may be possible to obtain information on the inheritance of parent's salt taste. Furthermore, it may be a basis for showing the relationship between daily salt intake and salty taste. In addition, test results may also be a source of dietary advice to prevent excessive salt intake with regard to daily salt intake.

In this study, we aimed to investigate the relationship between salt intake and salty taste by conducting taste tests on elementary school students and their parents through the analysis of salt intake based on measurement of urinary salt excretion and the use of Salsave® taste test papers (17). The same examination was also carried out on female high school students with no consideration of the parent-child relationship on a separate day, and the results were compared.

Methods

Subjects

Elementary school students and their biological parents. We recruited healthy volunteers who were assumed to meet the following conditions: 1) elementary school children aged from 10 to 12 years old, 2) the students' natural parents those who suffer from cardiovascular diseases, are healthy without history, 3) the student's biological parents do not have smoking or drinking habits, and 4) the students and their biological parents do not take any medicines. Twenty-six families (26 fathers, 26 mothers, and their 26 elementary school-aged children) who satisfied these criteria were the subjects of this study.

High school students. We recruited volunteers comprising 25 healthy female high school students in the second grade of Japanese high school. The examination was conducted on one day during a 50-minute class hour.

Ethics Committee

This research was approved by the Chiba Prefectural University of Health and Medical University (approval no. 2013-035). This study was performed from the 1st of February, 2014 to the 30 of April, 2015. All subjects were provided detailed information on the experiment, and they gave us written informed consent. This study was done in accordance with the Declaration of Helsinki.

Research and experimental procedures

Twenty-four-hour salt intake measured by urine salt exvretion. The amount of salt intake was estimated by the amount of urinary sodium excretion measured in a urine sample upon getting up. Urine test cups, urine test paper (Uro Paper Salt[®]; Eiken Chemical Co., Ltd.) (18), and the explanation of the urine test paper were distributed to the subjects the day before the test. The urine samples were collected from immediately after sleeping on the day before the salty taste test to the time of the first-voided urine specimen obtained on the morning of the test day. The total volume of the collected urine samples was measured with the urine test cup, and then the salt concentration of the urine sample was measured with the test paper. These data were recorded on paper at home by the subjects.

We calculated the estimated 24-hour daily salt intake by substituting the amount of urine measured with the urine test cup and the amount of salt obtained from the urine test paper according to the instructions of the urine test paper into the following equation (18): Estimated 24-hour salt intake (g/day) = 2.665× early morning (including nighttime) urine volume (ml) \times test paper's salt weight (g/L) + 4.206. Each subject's salty taste preference was measured by salt taste test papers (Salsave®, ADVANTEC Toyo Roshi Kaisha, Ltd.) (19). These commercially available salt taste test papers have salt concentrations of 0, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mg/cm² (17). Most healthy volunteers recognize a salty taste on the test paper at a concentration of 0.6 mg/cm², so test papers with a lower concentration threshold than that of 0.6 mg/cm^2 are required. Therefore, salt taste test papers with lowdensity threshold concentrations of 0.05, 0.1, 0.2, 0.3,

0.4, and 0.5 mg/cm² were specially ordered from AD-VANTEC Toyo Roshi Kaisha, Ltd. and used in conjunction with the commercially available product.

The salt taste test was carried out as follows. A test paper containing no salt (0 mg/cm²) was placed on the center of the tongue, and it was confirmed that the subject discerned no taste other than that of the filter paper. Then, a filter paper with a low salt concentration was placed on the center of the tip of the tongue, and the subject's mouth was closed for 3 seconds. The salt concentration at which some taste was sensed but could not be clearly recognized was defined as the minimum detection threshold, and the point at which a salty taste was clearly recognized was defined as the cognitive threshold. Measurement was continued with increasing salt concentration in order until saltiness was felt.

Food questionnaire

Subjects responded to food intake frequency survey on foods rich in salt using the waiting time of examination. The contents of the food intake frequency survey were as follows. 1) Estimation of household salt concentration by subjects, 2) Frequency of snacks (buying lunch boxes and shippers at stores, eating at home or work), 3) Frequency of eating out, 4) Frequency of drinking soup, 5) Frequency of eating noodles, udon, soba, 6) Amount to drink noodles juice, 7) Frequency of eating fried food, 8) Frequency of eating confectionery. Response of the food intake frequency questionnaire was as follows.

1) is 0: unknown, 1: 1 day 7 g or less, 2: 1 day 8 to 9 g, 3: 1 day 10 to 11 g, 4: 1 day 12 to 13 g, 5: 1 day 14 g or more. 2) - 6) and 8) are 0: None or almost none, 1: 1-2 times per week, 2: 3 times, 4 times per week, 3: 1 times per day, 4: 2 times per day or more. 7) is 0: do not drink, 1: 20 -30% drink, 2: about 50% drink, 3: about 70% drink, 4: mostly drink.

Statistical analysis

The target sample size was 25 for 1 group. It would provide at least 80% power.

In the actual analysis, all members of each group shown in Table 1 (for example, 26 children) were used

	Boys Girls		Children (boys+girls)	Mothers	Fathers	High school girls	
Age (years)	10.2 ± 1.4	10.4 ± 1.2	10.3 ± 1.3	40.7 ± 4.3	41.4 ± 7.5	16.9 ± 0.34	
Hight (cm)	139.7 ± 12.1	139.4 ± 7.9	139.5 ± 10.0	158.7 ± 5.9	167.2 ± 25.9	158.9 ± 4.79	
Weight (kg)	34.4 ± 9.3	32.2 ± 6.6	33.3 ± 8.0	53.8 ± 8.9	63.9 ± 13.9	52.8 ± 4.80	
Body mass index (kg/m ²)	17.3 ± 2.4	16.4 ± 2.3	16.9 ± 2.4	20.8 ± 4.5	22.3 ± 4.4	20.9 ± 1.91	

Table 1. Characteristics of the subjects

as subjects. Measured values are expressed as mean \pm standard deviation. Wilcoxon's inequality signed-rank test was used for comparison between the groups. Spearman's rank correlation coefficient was used to test the correlation between the salt taste thresholds (minimum detection threshold and cognitive threshold), the salt intake estimated from the nocturnal urine, and food questionnaire. The level of significance was set at 5%. SPSS for Windows (ver. 22J) was used for statistical analyses.

Results

The characteristics of the subjects are shown in Table 1. Twenty-six families (26 fathers, 26 mothers, and their 26 elementary school-aged children) who satisfied these criteria were the subjects of this study. High school students. 25 healthy female high school students in the second grade of Japanese high school.

The comparison of mean taste thresholds (minimum sensing threshold and recognized salt taste thresholds) and estimated 24 -hour salt intake are shown in Figure 1. The mean salt intakes (g/day) were 7.32 ± 1.63 in the children, 7.83 ± 2.30 in their mothers, and 9.05 ± 2.73 in their fathers. The mean minimum detection thresholds of some as yet unrecognized taste (mg/cm²) were 0.12 ± 0.23 in the children, 0.96 \pm 0.26 in their mothers, and 0.11 \pm 0.08 in their fathers. The mean cognitive thresholds, at which a salty taste was clearly recognized, (mg/ cm^2) were 0.28 ± 0.33 in the children, 0.33 ± 0.25 in their mothers, and 0.25 ± 0.14 in their fathers (Figure 1). The mean salt intake in the high school students was 8.52 ± 3.00 g/day, the minimum detection threshold was 0.13 ± 0.08 mg/cm², and the cognitive threshold was 0.28 ± 0.13 (Figure 1).

Table 2 shows the relationship between parents and children about salt-taste threshold and salt intake. In the parent-child comparisons, a highly positive and significant correlation was found between the children and their mothers for estimated salt intake (r = 0.46, P < 0.05) and in the relationship between the child's cognitive threshold and the estimated salt intake of the mother (r = 0.46, P <0.05). There was no statistically significant correlation between the estimated salt intake and the minimum detection threshold in any group.

Table 3 shows the relationship between estimated salt intake and the cognitive salt taste threshold. A significant positive correlation was found between the estimated salt intake and the cognitive threshold in the primary school children and high school students (elementary school children: r = 0.48, P < 0.05; high school students: r = 0.47, P < 0.05). In addition, when the estimated salt intake was corrected per kg of body weight, the relationship between the estimated salt intake and the cognitive threshold showed a significantly positive correlation between the elementary school students (r = 0.44) and the high school students (r = 0.39) (P < 0.05). There was no statistically significant correlation between estimated salt intake and cognitive threshold for each mother or father. There was a significant positive correlation between the cognitive thresholds and salt intake in the children and the high school students (p < 0.05), but no such significant positive correlation was found in the children's mothers and fathers.

The results of the food intake frequency survey are shown in Table 4. Significant correlations were found between the cognitive threshold of salt and the intake frequency of eating out for elementary school students and the frequency of intake of noodles (r = 0.42, r = 0.40, p < 0.05). Predictions of salt intake per day showed a significant positive correlation





Fig. 1. Comparison of mean taste thresholds (minimum sensing threshold and recognized salt taste thresholds) and estimated 24-hour salt intake. Measured values are expressed as mean \pm standard deviation. Wilcoxon's inequality signed rank test was used for comparison between the groups. The level of significance was set at 5%. *p<0.05, **p<0.01. a; vs Mothers, b; vs Fathers, c; vs High school girls.

Table 2. Relationship between parents and children about salt-taste threshold and salt inta

24h- Salt intake (g/kg)	Minimum detection threshold of some salt	Salt-taste cognitive threshold			
$\begin{array}{c c} \text{Mothers} & \underline{0.46^*} & \text{Boys} & \underline{0.26} \\ (n=14) & (n=14) & (n=14) \\ \hline & & & \\ 0.29 \end{array}$	Mothers -0.21 Boys Fathers 	Mothers $\stackrel{-0.02}{}$ Boys $\stackrel{0.59^{**}}{}$ Fathers 0.12			
Mothers $\stackrel{0.63^{*}}{$	Mothers -0.11 Girls -0.05 Fathers	Mothers -0.16 Girls -0.20 Fathers			
Mothers $\frac{0.46^{*}}{(n=26)}$ Children $\frac{0.36 (p = 0.07)}{Fathers}$ (n=26) (n=26) (n=26) 0.46*	Mothers 0.12 Children 0.30 Fathers	Mothers $\xrightarrow{0.19}$ Children $\xrightarrow{0.37 (p = 0.06)}$ Fathers			

Spearman's rank correlation coefficient was used to test for a the correlation between the salt taste threshold (minimum sensing threshold of some taste, salt-taste cognition) and the salt intake estimated from nocturnal urine. *p<0.05, **p<0.01.

	Mothers	Themselves	Fathers
Boys (n=14)	MT <u>-0.07</u> Salt intake <u>0.45</u> CT 	MT <u>-0.01</u> Salt intake CT 	MT -0.09 Salt intake-0.14 CT
Girls (n=12)	MT -0.16 Salt intake 0.10 CT	MT Salt intake CT	MT 0.24 Salt intake CT
Children (n=26)	MT -0.16 Salt intake 0.01 CT	MT 0.38 Salt intake CT	MT 0.24 Salt intake 0.26 CT
High school girls (n=25)		MT <u>0.20</u> Salt intake <u>0.47</u> [*] CT	

Table 3. Relationship	between salt-taste	threshold (minim	um sensing	threshold	of some	taste, sa	alt-taste (cognition)	and salt	intake
within an individual										

Spearman's rank correlation coefficient was used to test for a the correlation between the salt taste threshold (minimum sensing threshold of some taste, salt-taste cognition) and the salt intake estimated from nocturnal urine. *p<0.05, **p<0.01.

Salt intake; 24h- Salt intake (g/kg), MT; Minimum detection threshold of some salt, CT; Salt-taste cognitive threshold

between high school students and mothers (r = 0.48, r = 0.52, p <0.05).

Discussion

The commercially available salt taste test paper Salsave[®] contains seven kinds of salt concentrations: 0, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mg/cm² (19). These concentrations were devised for the measurement of people suffering from cardiovascular diseases such as hypertension (16). The salt concentration of Salsave[®] differs strictly depending on the amount of saliva absorbed into the test paper, but from previous reports, the concentrations of Salsave[®] of 0, 0.5, 0.6, and 1.0 mg/cm² were a relative match to salt concentrations of 0, 0.7, 0.9, and 1.4% (15-17).

The mean cognitive threshold of the awareness of salty taste in healthy adults (average age 55.3 \pm 10.9 years old) is reported to be 0.97 \pm 0.40% for men and $0.96 \pm 0.35\%$ for women, and that in healthy university students is $0.65 \pm 0.10\%$ for men and $0.63 \pm 0.06\%$ for women (17). This previous report estimated that about half of healthy men and women could recognize a salt taste corresponding to the 0.6 mg/cm² concentration on the test paper, and, in fact, 70% of the healthy male and female university students (mean age 21.1 ± 1.1 years old) tested recognized a salty taste at the salt concentration of 0.6% (17). From this report, it is assumed that for Salsave® test paper, healthy adults can recognize a concentration of about 0.5 mg/cm^2 , and, in fact, more than 70% of the tested subjects did recognize a salty taste at this threshold.

In the present study, the mean of cognitive threshold of salt taste was $0.28 \pm 0.33 \text{ mg/cm}^2$ in the elementary school students, $0.33 \pm 0.25 \text{ mg/cm}^2$ in their mothers, $0.25 \pm 0.14 \text{ mg/cm}^2$ in their fathers, and $0.28 \pm 0.13 \text{ mg/cm}^2$ in the high school students. The numbers of subjects with a cognitive threshold of $\leq 0.6 \text{ mg/cm}^2$ in the elementary school students, 25/26 (69%) in the ir mothers, 26/26 (100%) in their fathers, and 24/25 (96%) in the high school students.

These results indicate that it is difficult to examine the cognitive threshold of salt taste, salt intake, and salt taste relationships in young healthy persons when using the commercially available concentrations in Salsave[®], which is mainly used to assess hypertensive people (13- 15). Matsuzuki et al. (16) used Salsave[®] to study healthy elementary school students and their guardians. They found no relationship between the two groups, and one of the reasons may be that it was difficult to measure the threshold value at a low concentration range with a commercial product such as Salsave[®].

According to the report of Nishimoto et al. (19), who used test papers with the same low concentration thresholds as in the present study, a survey of the subjects with a mean age of 37.2 (21 to 70) years old showed that 20 of 30 people (0.4%), 6 (0.6%) people, and 4 (0.8%) people. However, the authors did not analyze the urinary sodium chloride excretion of their subjects', so the relationship between salt intake and salt taste in this study is unknown.

Antonello and colleagues (20) reported that there was a significant correlation (r = 0.37) between salt taste tests using NaCl concentrations of 0.228, 0.458, 0.913, 1.826, 3.652, 7.305, and 14.61 g/L and the amount of salt excretion in the urine of university students. The present study also found a significant correlation between the amount of salt excretion during urine storage and original salt taste test values. As well, after the estimated salt intake was corrected per kg of body weight, the correlation between salt intake and salty taste test values correlated significantly between the elementary school students and high school students (r = 0.42 for elementary school students and r = 0.39 for high school students), almost the same values as reported by Antonello et al.

The value of salt intake recommended by the Japanese Ministry of Health, Labor and Welfare is less than 10 g/day, the dietary intake standard is less than 7.0 g/day, and that of a reduced-salt diet used to treat hospital patients is less than 6 g/day. Although the average value of salt intake of Japanese people has been decreasing 1, in the present study, the mean salt intake of the elementary school students was 7.32 ± 1.63 g/day and that of their mothers was 7.83 ± 2.30 g/day. These values slightly exceed the meal intake standards of the Japanese Ministry of Health, Labor and Welfare, but the average values were even higher for the fathers (mean of 9.05 ± 2.73 g/day) and for the high school students (estimated mean salt intake of 8.52 ± 3.00 g/day). According to these figures, the standard deviation of the subjects in this study is large. For example, the maximum value for elementary school students was 10.6 g/day, and that for the high school students was 15.7 g/day, indicating that excessive salt consumption is still occurring. We also found that the cognitive threshold of a salty taste was significantly higher in the mothers, who frequently ingest soup and noodles, and in the elementary and high school students, who frequently eat out. These findings infer a relationship between the elevation of the cognitive threshold of a salty taste and eating habits in which foods with high amounts of sodium chloride are consumed.

It is known that taste thresholds increase with ageing (21). In the present study, we speculated that because a relationship between salt intake and the cognitive threshold rather than the minimum detection threshold was observed, the susceptibility to the habit of ingesting excessive amounts of salt over many years may be slowed down. However, this was unclear in the present research alone, and further study is necessary to determine whether changes in the thresholds of minimum detection and cognition are due to a genetic background and/or ageing.

From the viewpoint of the prevention of cardiovascular morbidity, the concentration of salt contained in snacks and in foods eaten out is generally high (6), taking into consideration the recent state of meals eaten and the frequency of eating out (7), from childhood or until a healthy person considers that ingesting the proper amount of salt is important. The test papers used in this research can be used to detect a low range of salt concentrations, and their accuracy was confirmed by Nishimoto et al. (19). Because this indicates that there is proper recognition of salt intake, it would seem that self-analysis would be easy to do so that people can receive nutritional guidance and nutrition education in the future. Furthermore, there was a significant correlation between the estimated salt intake of the mother and her child and between the estimated salt intake of the mother and the cognitive threshold of her child. This suggests that mothers and their elementary school children are still eating the same meals, indicating that it would be easy to educate mothers to achieve a reduction in the salt content of the meals they cook. Under current circumstances, due to low demand and the expense, test papers with low salt concentration ranges must be made to order, but considering the salty taste of young people and healthy people and the utility of the test papers as food education materials, wider use of such test papers is also desired.

This study has several limitations. Nocturnal urine was sampled but due to subjects' privacy considerations, urine volume was measured by the subjects with a urine test cup at home, so the accuracy of the measurements cannot be guaranteed. Among the subjects, salty taste discerned by the female high school students and the mothers of the elementary school students may have been influenced by the menstrual cycle, but the menstrual cycle in these subjects could not be aligned. In addition, to allow prompt examination of the elementary school students, testing was carried out first with the mother and father present. The order of use of the test papers was not made known to the subjects, and because of this, the proper answers to the taste test results of the parents were given in front of the child, and this might have influenced the accuracy of the results.3

Conclusion

The use of salt taste check papers to determine the cognitive salt taste threshold allowed for easy measurement of the salt taste threshold in young healthy individuals. The results suggested a relationship between daily salt intake and the subjects' salt taste threshold.

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