EFFECT OF REDUCING DIETARY SODIUM ON URINARY CALCIUM EXCRETION IN HEALTHY ADULTS FROM MAHIDOL UNIVERSITY, THAILAND

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ABSTRACT:

Background: There is evidence that not only an increasing sodium intake but also an inadequate dietary calcium intake can worsen the non-communicable diseases such as hypertension, osteoporosis, etc. Moreover, most people were less likely to maintain adequate calcium intake or to control sodium intake. Preventing calcium loss from the body along with sodium intake reduction, instead of controlling sodium intake alone or maintaining adequate calcium intake itself, could provide a better outcome in prevention and control of diet related health problems. This study aims to evaluate the effect of reducing dietary sodium on urinary calcium excretion in healthy subjects.

Methods: A single-group repeated measures design was used with twenty healthy adults from Mahidol University, Thailand for three experimental periods of three days each. Participants were provided three study diets; a normal standard recipe (100 percent sodium) followed by two experimental recipes with 30 percent reducing sodium content, one was added with 0.3 percent monosodium glutamate and another without adding monosodium glutamate in it. Dietary sodium and calcium intakes were evaluated form three-day food records whereas changes in urinary calcium excretion over three study phases were observed from spot morning urine samples taken at the end of each study period.

Results: There was a significant positive correlation between dietary sodium intake and urinary calcium excretion (r = 0.262, *P*-value = 0.043), or urinary calcium to creatinine ratio (r = 0.241, *P*-value = 0.032). Changes in urinary calcium excretion over three study periods were observed proportionately with changes in dietary sodium intake rather than that of calcium intake itself, and regression analysis indicated that every 1,000 mg/day increment in sodium intake could increase urine calcium loss by 8 mg/dL (*P*-value = 0.037) after adjusting for other dietary and lifestyle confounding factors.

Conclusion: The observation of lowering urinary calcium loss by consuming reduced sodium diets suggests a plausible mechanism through which dietary sodium reduction might lead to more favorable outcomes in the medical nutrition therapy for patients with hypertension, osteoporosis and other chronic non-communicable diseases. Furthermore, these results appear to support the “Thailand Salt Reduction Initiative Strategy”.

Keywords: Dietary sodium, Monosodium glutamate, Repeated measures, Spot morning urine, Urinary calcium excretion

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INTRODUCTION

In the past decades, almost all studies paid attention on the effect of dietary sodium on hypertension. Recently, calcium has been interesting and more than 20 epidemiological studies have reported that there is "an inverse relation between dietary calcium intake and blood pressure" [1]. Dietary calcium deficit may increase the circulating parathyroid hormone (PTH) level that leads to bone

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resorption (osteoporosis) and raises an intracellular free calcium level which stimulates the contraction of vascular smooth muscle and causes high blood pressure [2]. A salt-loading observational study in normotensive subjects reported that every 100 mmol (2,300 mg) of urinary sodium excretion leads to approximately 1 mmol (40 mg) of calcium loss in the urine [3]. Moreover, an increased sodium intake will increase intracellular free calcium level and both lead to high blood pressure [4].

According to the National Health Examination Survey (NHES - IV, 2008 - 2009), the average salt intake among Thais was 10.8 g/day. The prevalence of hypertension in Thai men and women were 22.0% and 21.4% respectively, and not decreasing enough in comparing with that of NHES – III, 2004 [5]. To date, salt consumption of Thai people would be more than the current World Health Organization (WHO) recommended level since almost all Thai dishes are rich in flavor and scrumptious by using salt and sodium containing condiments as main flavor enhancers. Hence, it is likely to be difficult for Thai people to follow low tasty foods using less flavor enhancers. Although all condiments are rich in sodium content, the amount of sodium in monosodium glutamate (MSG) is 12% that is approximately one third of those in sodium chloride. In addition, 0.2 – 0.8% of MSG has been used as a flavor enhancer in many clinical studies [6]. Using a proper amount of MSG in preparing foods could shorten 30 - 40% of sodium chloride without losing its savor [7]. Maintaining or improving appetite particularly for patients with chronic diseases is one of the imperative strategies in the dietary management for them.

Actually, 24-hr urine collection is the well-known acceptable and accurate method for determination of either renal creatinine clearance or dietary mineral intakes. However, it may be cumbersome for participants to collect the whole day and there may have somewhat substantial error due to incomplete collection, improper handling and processing method [8]. Kara, Erko and their colleagues [8] affirmed that there was a significant correlation between spot morning urine calcium to creatinine ratio and 24-hr urinary calcium excretion ($r = 0.521$, *P-value* < 0.01). Moreover, all former studies were salt-loading experimental studies and there was no previous study conducted with the reduced sodium diet (only 30 - 40% reducing sodium content of original recipe).

This study aims to evaluate the reduction of urinary calcium loss in healthy subjects by using the reduced sodium diets with 0.3% MSG and without MSG added in it. The combined effect of reducing urinary calcium loss with low sodium intake will benefit in the dietary management for the patients with hypertension and its related complications, and osteoporosis.

MATERIALS AND METHODS

Participant

This study was a pilot experiment and the estimated sample size was 12 subjects in minimum and 30 participants in maximum [9, 10]. The information about the research was announced with posters around the Mahidol University, Thailand. The inclusion criteria were participants aged between 18 – 45 years for both sexes who had never been diagnosed with any medical disease such as hypertension, renal disease, parathyroid disease, etc. They all were literates and willing to participate in this study. Participants would be excluded if they had a history of food allergy or monosodium glutamate sensitivity, they were currently taking any medication or vitamin and mineral supplements, current blood pressure higher than 140/90 mmHg, and menopausal women.

Ethical consideration

This study was approved by the Mahidol University Central Institutional Review Board on October 30, 2015 (COA No. MU-CIRB 2015/133.3010). The written informed consent was obtained from each participant after informing clearly the purpose of the study and ensured confidentiality for all information.

Methods

This study was conducted with a single-group repeated measures design [11] at the Institute of Nutrition, Mahidol University in December 2015, and lasted for two weeks. There were a baseline period and three experimental periods with three days each uninterruptedly. For baseline period, they consumed their own usual diets. After that, all participants were consumed the normal standard recipe with 100% sodium content for three days. Then, they were consumed 30% reduced sodium recipes with adding 0.3% MSG for second period, and without adding MSG for the last period accordingly. Foods were randomly provided from five food items as three-day rotating menus but different recipes. Three-day food records were taken for the baseline period to assess their usual intakes and three experimental periods respectively. Participants' spot morning urine samples were taken at the end of baseline period and each experimental period respectively for evaluation of urinary calcium excretion over three study periods.

Data collection and measures

Before starting the study, participants were asked with the questionnaires including general information (age, gender, religion and occupation), and physical activity and lifestyle information. Height and body weight were measured up to the nearest value of 0.1 cm and 0.1 kg respectively. Blood pressure was measured after resting at least 10 minutes by using digital sphygmomanometer (OMRON).

For all experimental foods, the preliminary sensory evaluation was performed to attain the foods that would be well-accepted by the participants. The same raw foods and ingredients were used throughout the study in order to minimize variation in nutrient contents. Foods were provided as three meals and snacks according to their estimated energy intake from baseline three-day food records. Participants consumed their breakfast, lunch and snacks at the research place, and dinner was provided as cooked foods together with snack in a package. Weekend meals were picked up on Friday with guideline instruction to keep and prepare foods at their home.

Participants were explained the detailed instruction with portion size since one week ahead of the study. They were requested to consume only the experimental foods offered and avoid extra-foods as much as they could. If they ate extra-foods or added any condiment to experimental foods, they were asked to note down as much detail as possible in the food records. Participant's compliance on diet was evaluated by reviewing their food records on the next day when they picked up the foods. By using INMUCAL-Nutrients V.3, calorie and nutrient intakes were calculated. All cooked food samples of three recipes were sent to the laboratory at the Institute of Nutrition, Mahidol University for chemical analysis of sodium and calcium content by using Atomic Absorption Spectrophotometry. These chemical analysis results were used throughout the study.

Each participant was provided a urine collection cup and an acid-washed urine container labelled with specific code-number. They were explained initially with written instruction how to collect spot urine sample correctly for each study period (approximately 30 ml urine without any contamination such as water, blood and lavatory paper etc.). For baseline period, they were asked to provide the spot morning urine sample before consuming the first breakfast meal of the standard period provided by the researcher [8]. Similarly, at the end of each experimental period, the corresponding spot morning urine samples for standard, MSG and without MSG periods were collected before having first breakfast meal of the subsequent period. Throughout the study, they collected the urine for four times.

They noted down urine collection time and date on the slip of the container. On the day of collection, the collected urine samples were sent to the accredited clinical laboratory for analysis of urinary calcium and creatinine concentration. Urinary creatinine was assessed by using Jaffe's method [12], and the quantitative determination of urinary calcium was performed by photometric assay on Roche/Hitachi cobas c system analyzer. The changes in absorbance were measured photometrically at 340 nm which is directly proportional to the concentration of calcium in urine [13].

Statistical analysis

Statistical analysis was done by using IBM ® SPSS software version 20. The normality of data's distribution for each variable was checked with Shapiro-Wilk test before doing further analyses. The changes in the urinary calcium excretion over three study periods were examined by repeated measure analysis of variance. The average of three days dietary sodium or calcium intakes and urinary calcium excretion at the end of each diet period were used to test the relation between dietary intakes and urinary excretion by using Spearman's correlation procedure. Stepwise multiple regression analysis was used to detect relation between urinary calcium excretion (a dependent variable) and the dietary intakes, anthropometric and lifestyle factors. *P-value* less than 0.05 was considered as statistically significant.

RESULTS

Twenty healthy volunteer adults were entitled as eligible subjects (11 staff and nine graduate students) from the Mahidol University, Thailand and they all accomplished the whole study period. All their baseline characteristics are described in Table 1.

Table 1 General characteristics of the participants at baseline

Variables	Total	Male	Female
Gender	20	3 (15)	17 (85)
Age (years)	31.1 ± 6.4	31.0 ± 4.4	31.1 ± 6.8
Systolic blood pressure (mmHg)*	117.6 ± 11.6	130.0 ± 3.0	115.4 ± 11.2
Diastolic blood pressure (mmHg)*	74.4 ± 6.5	81.0 ± 1.0	73.2 ± 6.4
Height (cm)*	159.4 ± 7.0	166.7 ± 2.9	158.1 ± 6.8
Weight (kg)	59.3 ± 10.9	59.8 ± 4.3	59.2 ± 11.8
BMI (kg/m^2)[¥]	23.4 ± 4.6	21.3 ± 1.5	23.7 ± 4.9
Underweight	2 (10)	-	2 (11.8)
Normal	9 (45)	2 (66.7)	7 (41.2)
Overweight and obese	9 (45)	1 (33.3)	8 (47.1)
Level of alcohol drinking [€]			
No drinker	12 (60)	0 (0)	12 (70.6)
Light drinker	8 (40)	3 (100.0)	5 (29.4)
Heavy drinker	-	-	-
Physical activity level			
Sedentary	17 (85)	2 (66.7)	15 (88.2)
Moderate (sport 3-5 times/week)	3 (15)	1 (33.3)	2 (11.8)
Vigorous (sport 6 -7 times/week)	-	-	-
Energy intake (kcal/day)	1382.1 ± 226.1	1403.7 ± 29.3	1378.3 ± 246.0
Carbohydrate (g/day)	193.2 ± 41.8	182.7 ± 36.3	195.0 ± 43.4
Fat (g/day)	43.7 ± 10.9	48.5 ± 14.3	42.9 ± 10.5
Protein (g/day)	53.7 ± 12.4	58.2 ± 17.4	52.9 ± 11.9
Sodium (mg/day)	2399.5 ± 933.3	1852.7 ± 588.2	2495.9 ± 961.9
Calcium (mg/day)	361.9 ± 175.1	257.5 ± 128.1	380.3 ± 178.8
Dietary fiber (g/day)	6.7 ± 1.8	5.5 ± 1.1	6.9 ± 1.9
Urinary creatinine excretion (mg/dL)	68.3 ± 38.8	73.5 ± 14.5	67.3 ± 41.9
Urinary calcium excretion (mg/dL)	6.4 ± 6.9	9.0 ± 7.7	5.9 ± 7.0
Urinary calcium to creatinine ratio	0.09 ± 0.08	0.12 ± 0.11	0.09 ± 0.07

Data are presented as mean ± SD or n (%)
[¥] WHO/ International Obesity Task Force (2000) BMI classification for Asian people
[€] Level of alcohol drinking: Light drinker = 1 drink occasionally; Moderate drinker = 1 drink/day for female and 2 drinks/day for male; Heavy drinker = more than 7 drinks/week for female and more than 14 drinks/week for male
Significant difference of variable between males and females (*P-value* < 0.05) determined by using (*) independent t-test for continuous data

Table 2 Changes in nutrient intakes of the subjects throughout three study periods

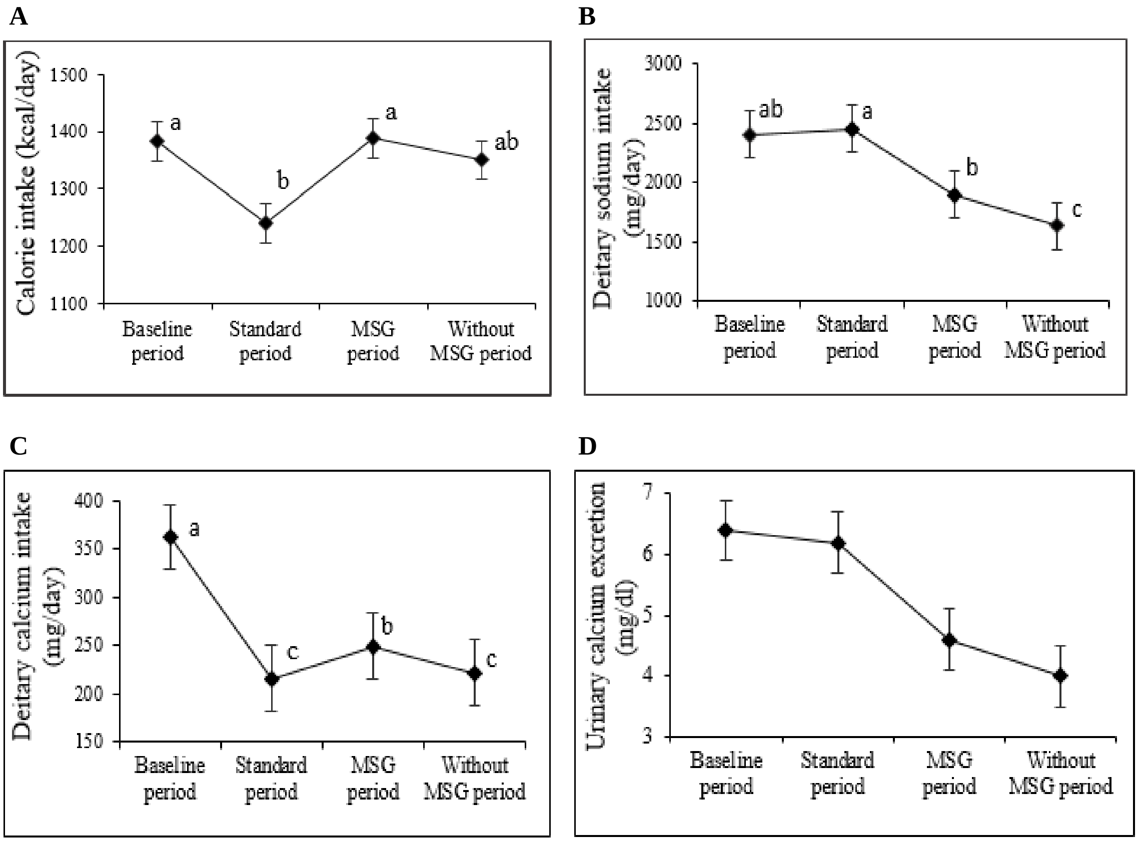
Variables	Experimental period		
	(1) Standard	**(2) MSG**	**(3) Without MSG**
Energy intake (kcal/day)	1240.9 ± 186.4^{b}	1388.2 ± 124.0^{a}	1350.1 ± 139.2^{ab}
Experimental food	1206.1 ± 170.8^{b}	1334.1 ± 114.6^{a}	1305.9 ± 133.3^{ab}
Extra foods consumed	34.9 ± 48.3	54.1 ± 54.6	44.3 ± 59.1
Carbohydrate (g/day)	200.3 ± 29.2^{b}	219.0 ± 19.6^{a}	213.1 ± 23.2^{a}
Fat (g/day)	29.3 ± 5.0^{b}	35.9 ± 3.7^{a}	34.9 ± 3.6^{a}
Protein (g/day)	44.6 ± 7.2^{b}	49.6 ± 4.6^{a}	48.2 ± 4.7^{a}
Sodium (mg/day)	2445.4 ± 401.3^{a}	1893.3 ± 185.6^{b}	1629.2 ± 192.3^{c}
Experimental food*	2402.8 ± 391.8^{a}	1856.8 ± 189.4^{b}	1599.6 ± 206.1^{c}
Extra foods consumed	42.6 ± 90.8	36.5 ± 72.1	29.6 ± 56.6
Calcium (mg/day)	215.4 ± 48.4^{b}	249.1 ± 27.6^{a}	221.6 ± 27.9^{b}
Experimental food*	200.4 ± 23.3^{b}	238.6 ± 21.6^{a}	211.9 ± 21.5^{b}
Extra foods consumed	14.9 ± 37.9	10.6 ± 15.3	9.7 ± 18.5
Dietary fiber (g/day)	10.5 ± 1.2^{b}	15.9 ± 1.8^{a}	15.8 ± 2.1^{a}

Data are presented as mean ± SD.
Calculation of nutrient intakes from the food records by INMUCAL-Nutrients V3
*Sodium and calcium intakes from the experimental foods calculated by chemical analysis results
Different superscripts (a, b, c) symbolize significant difference between study periods (P-value < 0.05), repeated measure analysis of variance.

Table 3 Changes in urinary parameters of the subjects throughout three study periods

Variables	Experimental period		
	(1) Standard	**(2) MSG**	**(3) Without MSG**
Urinary creatinine (mg/dL)	47.8 ± 18.0	50.1 ± 24.5	45.9 ± 22.5
Urinary calcium (mg/dL)	6.2 ± 6.9	4.6 ± 3.4	4.0 ± 4.4
Urine calcium to creatinine ratio	0.14 ± 0.16	0.11 ± 0.09	0.08 ± 0.06

Data are presented as mean ± SD, repeated measure analysis of variance



Data are presented as mean ± SEM

Different superscripts (a, b, c) symbolize significant difference between experimental periods (P-value < 0.05), repeated measure analysis of variance

Figure 1 Calorie intakes (A), dietary sodium intakes (B), dietary calcium intakes (C) and urinary calcium excretion (D) at each study period

There was no significant difference in mean age, weight and BMI between males and females. None of the participants was smoker, and all three male and five out of 17 females were light drinkers [14]. Almost all participants were reported of light or moderate daily physical activity [15]. According to their food records, all baseline calorie, sodium and calcium intakes, and urinary parameters were not significant difference between males and females. Urine creatinine result of all subjects and their urinary calcium or calcium to creatinine ratios were within normal range [9, 12, 16].

Changes in nutrient intakes throughout three experimental periods

Participants' nutrient intakes in each study period are described in Table 2. There were significant differences in average calorie intake, carbohydrate, fat, protein and dietary fiber consumptions between experimental periods (1) and (2), but not between study periods (2) and (3). Calorie as well as calcium intakes from the provided foods during MSG period were significantly higher than that of standard period. Mean dietary sodium and calcium intakes were different significantly

Table 4 Correlation between dietary sodium or calcium intakes and urinary parameters

Variables	Correlation (r)	*P-value*
Sodium intake and urinary calcium excretion	0.262	0.043*
Sodium intake and urinary calcium to creatinine ratio	0.241	0.032*
Calcium intake and urinary calcium excretion	0.324	0.012*
Calcium intake and urinary calcium to creatinine ratio	0.209	0.055

* Significant at *P-value* (< 0.05), Spearman's rank correlation

Table 5 Stepwise multiple regression analysis of dietary intakes€ and general characteristics‡ with urinary calcium excretion

Variables		R^2	*P-value*
Urinary calcium excretion (mg/dL) =	0.008 x Dietary sodium intake (mg/d)	0.40	0.037*

€ Dietary intake from experimental diet: calorie, carbohydrate, fat, protein, sodium, calcium and dietary fiber

‡ General characteristics: age, gender, body weight, physical activity level, and alcohol drinking

* Significant at *P-value* < 0.05, stepwise multiple regression analysis

between periods (1) and (2), as well as, between periods (2) and (3).

Changes in urinary parameters throughout three experimental periods

There was no significant difference in urinary creatinine excretion between experimental periods (Table 3) and all were within normal range [12]. Although a statistical significant difference in urinary calcium excretion was not detected, there were somewhat decreasing trends in urinary calcium excretion throughout three experimental periods (Figure 1).

Relation between dietary sodium or calcium intakes and urinary calcium excretion

Average dietary sodium and calcium intakes were calculated from three-day food records of each period. There were significant positive correlations between dietary sodium or calcium intakes and urinary calcium or calcium to creatinine ratio (Table 4).

Stepwise multiple regression analysis showed that dietary sodium intake accounted for 40% of the variance in urinary calcium loss and every 1,000 mg/day increment in sodium intake would increase urinary calcium excretion by 8 mg/dL (R^2 = 0.40, *P-value* = 0.037) after adjusting the confounding factors such as age, gender, physical activity level and lifestyle factors, and other dietary factors (Table 5).

DISCUSSION

In this study, the standard period could be assumed as run-in period for all subjects and his or her own control group. A possible carry-over effect from the former period was minimized by not taking urine sample on the first two days of each experimental period. Although 80% of participants consumed extra-foods besides the experimental diets, amount of extra-foods consumed were negligible and not different significantly over three study periods. In addition, approximately 97% of average calorie intakes, 98% of sodium intakes and 95% of calcium intakes were mainly come from experimental diets in each study period. Hence, this could be assumed that almost all nutrient intakes mainly came from the experimental diets.

When starting the standard diet, participants' average calorie intake was reduced significantly from baseline usual intake by 141.2 $\pm$ 295.1 kcal/d and also calcium intake was reduced by 146.6 $\pm$ 186.9 mg/d (Figure 1). This might be due to the sudden changes of their usual meal pattern and they were not easily accustomed to the study diets, although all standard foods were prepared with common regular Thai dishes. However, their sodium intake was not different or even slightly increased than their baseline. This might be somewhat under-estimation of their baseline food records in terms of recording Na intake since most of the participants consumed their usual meals at outside and could not estimate exactly the amount of sodium in foods they ate.

However, beginning from the second experimental period, their calorie intakes were increased as nearly the same as their baseline intake. It might be due to the umami taste of MSG in foods [7], and this was consistent with the findings of Walker R and Lupien JR, they reported that 0.2 – 0.8% of MSG usage in preparing foods gave the finest palatability for human beings [6]. Despite average calorie and calcium intakes in MSG period were significantly increased, their average sodium intake was still significantly lower than that of standard period by 545.9 ± 355.9 mg/day.

One more comparison between MSG and without MSG periods showed that their average calorie intake was lower in latter period along with significantly reducing in both sodium and calcium intakes. These changes were nearly the same whether extra-foods consumed were excluded or not in the analysis. Throughout the study, their average sodium intake was not exceeded over 2,500 mg/day in the standard period, and those in both second and third periods were less than 2,000 mg/day which was totally agreed with the WHO recommended level of sodium intake for adults.

Participants' average compliance were 89%, 90% and 85% with study diet (1), (2) and (3) respectively which were calculated based on the amount of leftover-food per serving. In fact, participants consumed the experimental diet of MSG recipes more than the rest two recipes. According to their calorie intake results and compliance with provided foods, the acceptability of MSG recipe was said to be attained as nearly the same as standard recipe by reducing approximately 30% of sodium content without losing its savor [7].

There were significant positive correlations between dietary sodium intake and urinary calcium excretion or urinary calcium to creatinine ratio, as well as, calcium intake and urinary calcium excretion. Although this current study used spot urine, the correlation result was still consistent with the previous study conducted in Irish population where they found a significant positive correlation between sodium intake and 24-hr urinary calcium excretion (r = 0.56, *P-value* < 0.001 for males and r = 0.35, *P-value* < 0.01 for females) [17]. Furthermore, this was agreed with one large population study conducted in healthy Japanese adults where they observed 24-hr urinary calcium excretion was significantly correlated with daily sodium intake (r = 0.161, *P-value* < 0.05 for males and r = 0.213, *P-value* < 0.01 for females) [18].

Moreover, stepwise multiple regression analysis of the current study showed that sodium intake increased by 1,000 mg/day would increase urinary calcium excretion by 8 mg/dL after adjusting other confounding factors. This was consistent with findings of Itoh R and Suyama Y, they stated that urinary calcium excretion was increased by 0.6 mmol (24 mg) for an increase in 100 mmol (2,300 mg) of sodium intake (*P-value* < 0.001) [18]. Therefore, this current study could affirm that there is a positive relationship between sodium intake and urinary calcium excretion whether spot urine or 24-hr urine sample was observed. The statement that urinary calcium excretion is strongly influenced by dietary sodium intake is reasonable because reabsorption of urinary calcium is parallel with reabsorption of sodium at renal tubules [19].

Despite a statistical significant difference in urinary calcium excretion was not detected, there were somewhat decreasing trends in urinary calcium excretion over three experimental periods. That decreasing trend of calcium excretion was parallel with the decreasing trend of dietary sodium intake, but not with the pattern of calcium intake. Sodium intakes in MSG and without MSG periods were reduced by 22.6% and 33.4% respectively in comparing with that of standard period. Similarly, urinary calcium excretions were reduced by 25.1% in MSG period and 35.4% in without MSG period while comparing with that in standard one. Overall, these results implied that positive influence of reducing dietary sodium on lowering urinary calcium loss was independent on the level of dietary calcium intake as consumed in this current study. This was consistent with the findings of Castenmiller, et al. [9].

In order to assess the exact magnitude of dietary sodium effect on urinary calcium excretion, sodium intake should have been validated with 24-hr urinary sodium excretion [20], and evaluated with 24-hr urine calcium. Moreover, in order to see a statistical significant difference in urinary calcium excretion, the average sodium difference between the study diets should be approximately 100 mmol (2,300 mg) [3]. However, it was unbearable to study with this too extreme sodium diets in the general population. Other limitations of this study were unequal distribution of sex, short duration of the study and could conduct only with the participants from Mahidol University. Further studies are warranted to evaluate the acceptability of 0.3% MSG usage in the proper low sodium diets (140 mg or less of sodium per serving) among the general population with equal sex distribution.

CONCLUSION

There was a significant positive correlation between dietary sodium intake and urinary calcium excretion, and every 1,000 mg/day increment in sodium intake could increase urine calcium loss by 8 mg/dL. This could be detected without any burdensome to the participants by using spot urine sample. The acceptability of MSG recipe was said to be attained as nearly the same as standard recipe by reducing approximately 30% of sodium content without losing its savor. The combined effect of reduced sodium intake with decreasing urinary calcium loss may be benefit in the dietary management for patients with hypertension and its related complications, and osteoporosis patients as well as improving the palatability of these patients and elderly people. Furthermore, this study could contribute to "Thailand Salt Reduction Strategy" of the government policy.

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