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Increased Calcium Excretion in Urine by High Salt Diet Intake in Man and Rat

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ABSTRACT

Effects of sodium chloride intake on the metabolism of calcium and other minerals have been studied in man and rat. In the human experiment, 5 healthy subjects were fed the experimental diet with high salt or low salt content. Subjects ingested 20 g of NaCl a day during a high salt diet period and 4 g NaCl during a low salt diet period. The nutrient composition except NaCl content of the experimental diets was the same between the high salt and the low salt diets. Each of the dietary manipulation was continued for 9 successive days. The high salt diet intake increased urinary excretion of sodium and calcium, but had almost no effect on urinary excretion of other minerals. Since food intake except NaCl of all the subjects was the same between the high salt diet and the low salt diet periods, the increased calcium excretion was ascribed to the excess salt intake. Two experiments using the experimental animals were conducted in this study. In one of the experiments, rats were divided into three diet groups: control (2% NaCl) diet, low salt (0.5% NaCl) diet, and high salt (8%) diet groups. Each group of rats were meal-fed the corresponding diet for 14 days until the final day of the experiment. Food consumption of the rats was isoenergetically adjusted among three diet groups. The excretion of sodium and calcium in urine was greater in the high salt diet group of rats than in the other diet groups. The retention rate of calcium was significantly lower in the high salt diet group of rats than in the other groups. However, there was no difference in the excretion and retention rates of calcium between the control diet and the low salt diet groups, indicating that excess salt intake beyond a certain amount promotes excess calcium excretion in urine. In the other animal experiment, rats were fed a low salt (0.05% NaCl) diet or a high salt (5% NaCl) diet for 26 days, and half of the rats in each diet group were trained by voluntary exercise for 14 days starting on the day 10 in the experimental period. Following the exercise period, urine was collected for 2 days. It was confirmed that the high salt diet intake promoted urinary calcium excretion in sedentary rats. On the other hand, the calcium excretion in rats fed the high salt diet was significantly decreased by the exercise training, suggesting that the exercise training enhanced calcium utilization in rats. In the present study, it has been clearly demonstrated that high salt diet intake resulted in increased urinary calcium excretion in man and rat, and that the exercise training suppressed the calcium excretion promoted by high salt intake.

INTRODUCTION

Salt is an important nutrient for man and animals and a foodstuff giving flavor to foods. However, it is suggested that a high salt intake be avoided due to its deteriorative effects so far reported. One effect is that increased NaCl ingestion elevates the urinary calcium excretion in man and animals (Goulding, 1980; Breslau et al., 1982; Muldowney et al., 1982; Fujita et al., 1984; Goulding and Campbell, 1984; Shortt et al., 1988; Zarkadas et al., 1989). Lemann et al. (1979) concluded, however, that dietary sodium manipulation within more usual limits resulted in relatively small changes in urinary calcium. In the present study, we examined the effect of high salt intake on urinary calcium excretion in man and rat under conditions in which food intake was precisely controlled. Furthermore, effect of exercise training on the calcium excretion in rats fed a high salt diet was also examined.

METHODS

Experiment 1: human study.

Five healthy male students were used as subjects in this experiment. Characteristics of the subjects are given in Table 1. The experiment was conducted for 18 days consisting of 9-day low salt diet period

TABLE 1

Experiment 1: characteristics of the subjects

Number of subjects		5
Sex		Male
Age (year)		21.4±0.9
Height (cm)		174.0±1.5
Weight (kg):	Initial day	69.0±1.9
	Final day	68.0±1.7
Low salt diet	Initial day	69.0±1.8
	Final day	68.0±1.8
High salt diet	Initial day	68.7±1.9
	Final day	68.2±1.8
Blood pressure (day 9)		
Low salt diet	Diastolic	66±1
	Systolic	108±1
High salt diet	Diastolic	68±1
	Systolic	107±2

Values are means ± SEM.

and 9-day high salt diet period. A low salt diet contained 4 g of sodium chloride and a high salt diet 20 g of sodium chloride. The experimental diet provided 11, 68, and 21% of calories, as protein, carbohydrate, and fat, respectively. Three of the 5 subjects were given the high salt diet in the first 9-day period, and then the diet was switched to the low salt diet in the second 9-day period. The other 2 subjects were given each diet in a reverse pattern. The subjects were fed only the experimental diet during the period, but water was available ad libitum. The initial 3 days in each 9-day period were the duration for adaptation to each diet. Therefore, urine was collected for the following 6 days in each period. In a preliminary experiment, it was confirmed that 3 days were long enough for the subjects to adapt to each diet with respect to mineral excretion in urine. Data of urinary mineral excretion (Table 3) were expressed as mean values of the 6 days when urine was collected. The mineral content in urine was determined by atomic absorption spectroscopy using a Plasma Atomcomp (model 975, Jarrell-Ash, Boston). The experimental procedure followed conformed to the Helsinki Declaration of 1975 as revised 1983.

Experiment 2: animal study.

1. Effect of salt intake on mineral absorption and retention

Thirty male Sprague–Dawley rats (4 weeks old) were obtained from CLEA Japan, Tokyo. The animals were housed at 23°C with light from 0700 to 1900 h and were meal-fed a nonpurified commercial diet (CE-2, CLEA Japan) at 0900–1100 h and 1700–1900 h for 2 weeks. Then, the animals were divided into three groups; control diet, low salt diet, and high salt diet groups. A control diet, low salt diet, and high salt diet contained 2, 0.5, and 8% NaCl, respectively. The composition of each diet is given in Table 2. Rats were meal-fed the appropriate diet for 2 weeks. Food consumption of the rats was isoenergetically adjusted among the groups. Water was available ad libitum. Urine and feces were collected on the day 4, 7, and 10 in the period, and the data (Table 4) were expressed as the mean values of the 3 days. On the final day of the experiment, all of the rats were killed by decapitation to obtain serum. Mineral contents in urine, feces, and serum were determined by atomic absorption spectroscopy.

Mineral absorption and retention were calculated by the following equations.

TABLE 2

Experiment 2: composition of the diets for rats

Ingredient	Diet for rats		
	Low salt diet	Control diet	High salt diet
	(g/kg diet)		
Corn starch	400.0	400.0	400.0
Sucrose	172.7	157.7	97.7
Casein	250.0	250.0	250.0
Soybean oil	50.0	50.0	50.0
Cellulose	50.0	50.0	50.0
Vitamin mixture	12.5	12.5	12.5
Mineral mixture	64.8	79.8	139.8
(NaCl)	(5.0)	(20.0)	(80.0)
Mineral	(% in mineral mixture)		
NaCl	7.7	25.1	57.2
$CaCO_3$	36.1	29.3	16.7
KH_2PO_4	42.3	34.3	19.6
$MgSO_4 \cdot 7H_2O$	12.3	10.0	5.7
$CaHPO_4 \cdot 2H_2O$	0.52	0.43	0.24
$Fe(C_6H_5O_7) \cdot 5H_2O$	0.77	0.62	0.36
$MnSO_4 \cdot H_2O$	0.15	0.12	0.07
$ZnCl_2$	0.030	0.020	0.010
$CuSO_4 \cdot 5H_2O$	0.185	0.156	0.086
KI	0.0006	0.0005	0.0003
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.0031	0.0025	0.0014
$NaSeO_3 \cdot 5H_2O$	0.0018	0.0015	0.0009

Absorption = Intake – Fecal excretion
Absorption rate (%) = Absorption / Intake × 100
Retention = Absorption – Urinary excretion
Retention rate (%) = Retention / Absorption × 100

2. Effect of exercise training on calcium excretion in rats fed a high salt diet

Twenty six male Sprague-Dawley rats (6 weeks old) were used in this experiment. The animals were divided into two groups: low salt diet and high salt diet groups. A low salt diet contained 0.05% NaCl, and a high salt diet 5% NaCl. Other composition of each diet was the same as that given in Table 2. Food consumption was isoenergetically adjusted between two groups by the meal-feeding method (feeding at 0700–0900 h and 1900–2100 h) throughout the experimental period. After 4 days of feeding the non-purified commercial diet, each group of rats were fed the appropriate experimental diet, high salt diet or low salt diet, for 26 days. In this period, half of rats in each group were trained by voluntary exercise for 2 weeks starting on the day 9 in the experimental diet period. The exercise training was performed only during the dark period of a day (2100–0700 h) using a cage with a running wheel (37 cm of diameter). Urine was collected for 2 days just after the training period. Rats were not exercised during the 2 days. Urinary minerals were analyzed as described above.

RESULTS AND DISCUSSION

Experiment 1: human study

Body weight and blood pressure were not affected by intake of the high salt diet (Table 1). Concentrations of serum minerals (data not shown) and urine volume (Table 3) were no different between the two diet conditions. These findings indicate that intake of the high salt diet containing 20 g of NaCl did not give any acute effect to the metabolic conditions of the subjects. In fact, this amount of salt (20 g/day) is usually consumed by people in some area of Japan (Komachi, 1981).

TABLE 3
Experiment 1: urinary mineral excretion

	Low salt diet (n = 5)	High salt diet (n = 5)
Urine volume (l/day)	1.06±0.03	1.14±0.06
Na (mg/day)	971±72	6471±352*
Ca (mg/day)	102±7	150±13*
P (mg/day)	965±28	903±41
Mg (mg/day)	94±4	96±4
K (mg/day)	2186±122	1986±47

Values are means±SEM. *P <0.05.

Urinary sodium excretion was markedly increased by the high salt diet intake (Table 3). Urinary calcium excretion was also significantly increased to 1.5-fold by the high salt diet intake, whereas excretion of other minerals in urine was not affected by the diet (Table 3). In this experiment, the subjects were fed the controlled diet, and the NaCl content was only different between the high salt diet and the low salt diet. Therefore, it can be concluded that the high salt content in the diet is responsible for the promotion of urinary calcium excretion. This finding suggests that chronic intake of the high salt diet is involved in the risk factors in the development of osteoporosis.

Experiment 2: animal study

1. Effect of salt intake on mineral absorption and retention in rat

Mineral intake and excretion in rat are given in Table 4. Sodium excretion in urine was increased with increasing in salt intake. The absorption rate of sodium was slightly lower in the low salt diet group than in other two groups. On the other hand, the retention rate was higher in the low salt diet group than in the high salt diet group.

Although calcium intake was almost the same among three groups, urinary calcium excretion was markedly greater in the high salt diet group than in other two groups, resulting in lower retention rate of calcium in the high salt diet group. It may be noted that urinary calcium excretion was not different between the control and the low salt diet group, indicating that excess salt intake beyond a certain amount promotes calcium excretion in urine.

Phosphorus and magnesium excretion in urine were greater in the high salt diet group (P, 67 mg/day; Mg, 5.3 mg/day) than in the other two groups as well (P, 42–45 mg/day; Mg, 1.9–2.1 mg/day).

Potassium excretion in urine was slightly greater in the high salt diet group than other two groups. The high salt diet intake had a relatively smaller effect on potassium metabolism than on other mineral metabolism.

An average value of initial body weights of rats was approximately 200 g. The body weight on the final day of the experiment was slightly lower in the high salt diet group (271±2 g, mean±SEM) than in other two groups (control group, 286±4 g; low salt diet group, 290±3 g).

Although the serum sodium concentration was not affected by the high salt diet intake, the concen-

TABLE 4

Experiment 2: mineral intake and excretion in rats

	Low salt diet (n = 10)	Control diet (n = 10)	High salt diet (n = 10)
Na			
Intake (mg/day)	35.3±0.5a	142.1±1.9b	573.7±8.6c
Fecal excretion (mg/day)	2.2±0.2a	3.4±0.2b	3.6±0.4b
Urinary excretion (mg/day)	28.0±0.7a	122.4±3.5b	530.2±10.0c
Absorption (mg/day)	33.1±0.6a	138.7±2.0b	570.1±8.7c
Retention (mg/day)	5.1±0.8a	16.3±3.3b	39.9±6.4c
Absorption rate (%)	93.6±0.5a	97.6±0.2b	99.4±0.1b
Retention rate (%)	14.9±2.2a	11.7±2.4a,b	7.0±1.1b
Ca			
Intake (mg/day)	170.1±2.5	171.5±1.3	173.2±2.6
Fecal excretion (mg/day)	102.3±3.3	103.4±2.4	106.1±3.0
Urinary excretion (mg/day)	0.6±0.1a	0.7±0.1a	5.7±0.4b
Absorption (mg/day)	67.8±3.4	68.1±3.0	67.1±3.4
Retention (mg/day)	67.2±3.4	67.4±3.0	61.4±3.3
Absorption rate (%)	39.8±1.8	39.5±1.5	38.6±1.7
Retention rate (%)	99.0±0.1a	98.9±0.1a	90.8±0.9b
K			
Intake (mg/day)	140.9±2.1	142.1±0.9	143.5±2.2
Fecal excretion (mg/day)	2.4±0.3a	1.7±0.2a,b	1.5±0.2b
Urinary excretion (mg/day)	59.1±1.0a	58.7±1.7a	64.3±1.3b
Absorption (mg/day)	138.5±2.2	140.4±1.9	142.0±2.2
Retention (mg/day)	79.2±1.7	81.7±1.8	77.7±1.7
Absorption rate (%)	98.2±0.2a	98.8±0.1a,b	99.0±0.2b
Retention rate (%)	56.8±0.6a,b	57.8±1.1a	54.6±0.7b

Values are means±SEM. Means not followed by the same letters are significantly different ($P < 0.05$).

TABLE 5

Experiment 2: serum mineral concentrations in rats fed the control, low salt, or high salt diet

Mineral	Low salt diet (n = 10)	Control diet (n = 10)	High salt diet (n = 10)
Na (mg/ml)	2.5±0.0a	2.3±0.0b	2.6±0.0a
Ca (μg/ml)	105±1a	106±1a	96±1b
P (μg/ml)	161±3a	156±3a	135±3b
Mg (μg/ml)	18.7±0.3a	18.6±0.3a	14.6±0.5b
K (μg/ml)	102±2a	121±4b	110±3a,b

Values are means±SEM. Means not followed by the same letters are significantly different ($P < 0.05$).

trations of calcium, phosphorus, and magnesium were lower in the high salt diet group than in the other groups, reflecting higher excretion of these minerals in the high salt diet group (Table 5).

2. Effect of exercise training on calcium excretion in rats fed a high salt diet

Voluntary exercise activities of rats in the high salt diet and the low salt diet groups were recorded for 2 weeks (Fig. 1). The running activities in both groups were gradually increased during the period. Although the activity was not significantly different between two groups, there was a trend that the activity during the latter half of the period was higher in the high salt diet group than in the low salt diet group. Urinary mineral excretion was measured for 2 days just after the training period. The exercise training had no effect on urinary sodium excretion (Fig. 2). It was confirmed in this experiment that urinary calcium excretion in sedentary rats was promoted by high salt diet intake (Fig. 2). On the other hand, the calcium excretion in the high salt diet group was significantly lower in the trained rats than in the sedentary rats (Fig. 2), suggesting that

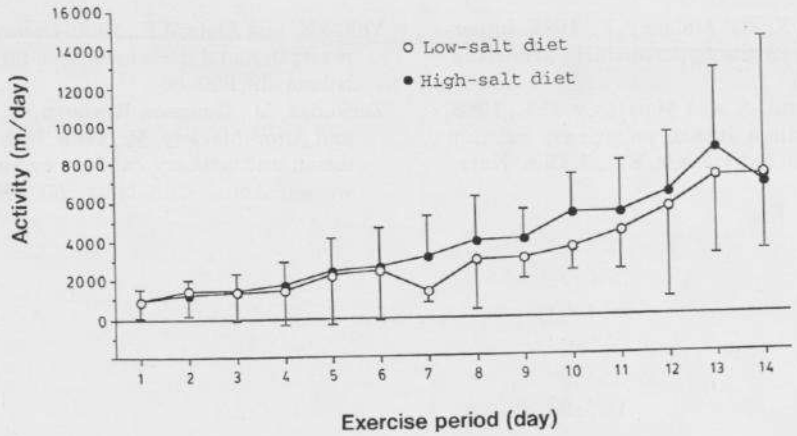

Fig. 1. The voluntary running activity of rat. Each point is a mean value with SEM.

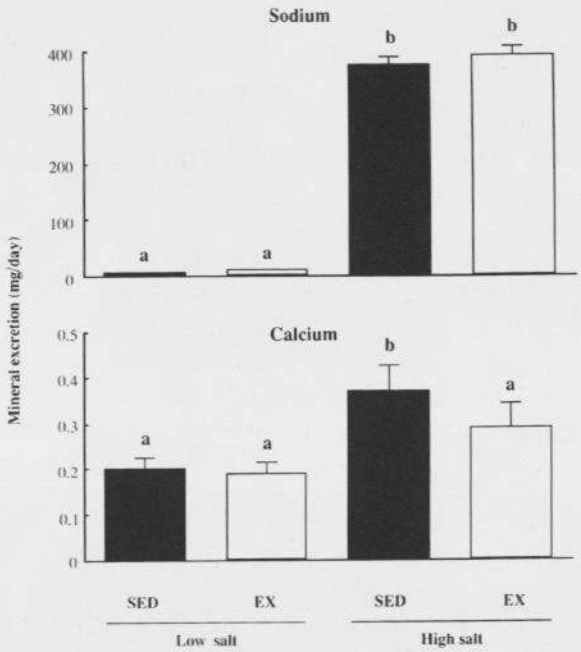

Fig. 2. Sodium and calcium excretion in rat urine. Values are means±SEM for 6–7 rats. Means not followed by the same letters are significantly different ($p < 0.05$).

the exercise training enhanced calcium utilization in rats and thus lowered urinary calcium excretion. It has been reported that exercise increases bone formation in rat (Yeh and Aloia, 1990), being consistent with our findings.

CONCLUSIONS

It has been clearly demonstrated that high salt diet intake promotes urinary calcium excretion in man and rat. Food intake, except the amount of NaCl, was precisely adjusted between/among experimental groups in this study, therefore the increased calcium excretion in urine was ascribed to the increased NaCl ingestion. The exercise activity suppressed urinary calcium excretion in rats fed the high salt diet. These findings suggest that high salt intake is one of the risk factors in the development of osteoporosis, and that exercise ameliorates the effect of high salt intake.

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