Negative Balance of Calcium and Magnesium under Relatively Low Sodium Intake in Humans

Naoko KODAMA, Mamoru NISHIMUTA* and Kazumasa SUZUKI

Division of Human Nutrition, The National Institute of Health and Nutrition, Tokyo 162–8636, Japan

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Summary The balance of minerals (sodium [Na], potassium [K], calcium [Ca], and magnesium [Mg]) was measured in six female students for 10 d while under a relatively low Na intake (100 mmol/d or 2.2 g/d) with receiving adequate Ca (20 mmol/d or 800 mg/d) and Mg (12 mmol/d or 280 mg/d). Both the plasma renin activity (PRA) and aldosterone level were above the reference ranges throughout the experiment, which implied that the subjects were Na deficient. However, the urine Na excretion was about the same as that ingested, while there was no substantial reduction of sweat Na concentration observed during moderate physical exercise (13.2 ± 2.6 mmol/L) (mean $\pm$ SD). On the other hand, the urine Ca and Mg levels were high, but the apparent absorption of Ca and Mg was moderate ($21 \pm 5\%$, $34 \pm 4\%$, respectively), which resulted in a negative balance of these two elements. It seems that the stored Na in the bone is eluted so as to compensate for the low dietary Na intake, while any excess Ca and Mg also inevitably flows into the blood stream with Na, which inhibited the intestinal absorption of both Ca and Mg and accelerates their excretion in urine.

Key Words balance, sodium, calcium, magnesium, human

In our previous study (1), unexpected results were demonstrated in the arm sweat contents of sodium (Na), calcium (Ca), and magnesium (Mg) of young Japanese females during relatively heavy bicycle ergometer exercise (1.5 kp, 50 rpm, 66 min, twice a day) while taking a relatively low mineral diet with dietary Na of 100 mmol/d (or sodium chloride 6 g/d).

The sweat Na was relatively lower, but the sweat Ca and Mg were obviously higher during the experiment as compared to identical experiments in which the dietary Na was 170 mmol/d (or sodium chloride 10 g/d) (2).

Although no reasonable hypothesis has yet been made to explain these results, one assumption has been proposed (3). Regarding the aspect of nutrition, these three minerals are all stored in the bone (4). Therefore, when any of these minerals reaches an insufficient level in the body, it is eluted from the bone to compensate for any shortage. The mechanism of elution of these minerals from the bone was reported to occur through non-mineral selective osteolysis by the macrophages (5).

If this mechanism occurs under Na restriction, then excess Ca and Mg may also be eluted along with the Na, and thus flow into the blood stream, and thereby inevitably cause a reduction of intestinal absorption as well as an increase in the urine excretion of these minerals.

The aim of this study was to measure the balance of minerals (Na, K, Ca, and Mg) as well as to evaluate the hormones affecting the Na metabolism in humans under a low Na intake.

SUBJECTS AND METHODS

This study was carried out according to the rules of the Helsinki Declaration, and was approved by the ethical committee of the National Institute of Health and Nutrition, Tokyo. A 17-d metabolic study, including two successive balance sessions of 5 d, was designed as shown in Table 1. Six female students took part in this study after giving their written informed consent and also receiving a full explanation of the purpose and methods of this experiment (Table 2). After examination of the first blood specimen, all subjects were regarded to be in good health. The subjects reported to the metabolic ward at the National Institute of Health and Nutrition in the afternoon before supper on day 1 and ate as much as they desired; they then went to bed at the scheduled time of 10:00 p.m.

During the period, starting from 6:00 a.m. on day 2 and ending at 8:30 a.m. on day 17, urine specimens were collected, and the 24-h urine and/or partition urine (on days 2, 7 and 12) excretion of minerals (Na, K, Ca, and Mg) was measured. On the morning of days 4, 9, and 14, the fasting morning blood was sampled, and the subjects also orally took a coloring marker for their feces (Carmine 0.5 g; Merk KGaA, Germany) just before breakfast.

Three subjects (a, c, e) pedaled a bicycle ergometer for 60 min once a day at the subject's selected intensity

* To whom correspondence should be addressed.

E-mail: nisimuta@nih.go.jp

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Table 1. Experimental design.

Experiment day		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Session		Pre			Balance I					Balance II					Post			
Diet menu			4	5		1	2	3	4	5		1	2	3	4	5		
Blood					○					○					○			
Fecal marker					○					○					○			
Urine	24h		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	Partition		○					○				○						
Sweat (Exercise)	Group A				○	○	○	○	○									
	Group B									○	○	○	○	○				

6:308:3012:3016:3018:3022:00

RiseBreakfastLunchSupperLights out

HeightWeightBP(Exercise)(SFT)

24h or partition urine

BP, blood pressure; SFT, skin fold thickness.

Table 2. Characteristics of subjects.

ID	Gender	Age (y)	Height (cm)		Weight (kg)		SBP (mmHg)		DBP (mmHg)		SFT (mm) ¹		Hb (g/dL)	
			First	Last	First	Last	First	Last	First	Last	First	Last	First	Last
Group A	a	f	22	160.0 160.4	54.34 53.21		118 102		68 58		34.5 32.5		12.5 12.5	
	c	f	21	163.8 163.9	57.74 57.33		124 106		80 68		38.0 32.0		14.6 14.2	
	e	f	22	143.3 144.6	51.63 51.41		104 104		68 56		55.0 50.5		13.0 12.9	
Group B	b	f	21	158.1 158.1	57.74 57.56		96 90		58 60		27.0 26.5		13.3 13.0	
	d	f	18	156.8 155.7	52.98 53.59		140 110		64 60		44.0 40.5		13.1 12.4	
	f	f	19	154.0 155.4	51.21 50.68		100 100		60 58		32.5 27.5		13.7 13.3	
Mean		21	156.0 156.4	54.27 53.96	114 102		66 60		38.5 34.9		13.4 13.1			
SD		2	7.0 6.6	2.90 2.91	17 7		8 4		9.9 9.1		0.7 0.7			
p			0.199		0.119		0.030*		0.023*		0.004**		0.013*	

ID	Gender	Age (y)	TP (g/dL)		Glucose (mg/dL)		T. Chol (mg/dL)		HDL-C (mg/dL)		TG (mg/dL)		Urate (mg/dL)	
			First	Last	First	Last	First	Last	First	Last	First	Last	First	Last
Group A	a	f	22	7.0 7.0	91 83		187 189		64 64		49 58		5.1 4.6	
	c	f	21	6.8 6.9	92 87		180 180		59 57		56 43		6.2 6.0	
	e	f	22	6.5 6.8	93 87		168 161		49 49		68 55		5.7 5.7	
Group B	b	f	21	7.3 7.3	86 88		186 173		53 55		75 47		4.7 5.3	
	d	f	18	7.3 7.1	92 77		194 191		66 64		59 40		3.9 4.3	
	f	f	19	6.9 7.1	89 85		183 172		62 65		43 34		4.1 4.2	
Mean		21	7.0 7.0	91 85	183 178		59 59		58 46		5.0 5.0			
SD		2	0.3 0.2	3 4	9 11		7 6		12 9		0.9 0.8			
p			0.197		0.023*		0.041*		0.425		0.030*		0.349	

The data obtained at first and last specimen are shown.
¹ SFT, skin fold thickness (sum of upper arm back and subscapla); S(D)BP, systolic (diastolic) blood pressure; Hb, hemoglobin concentration; TP, total protein; T. Chol, total cholesterol; HDL-C, HDL cholesterol; TG, triglyceride.
p*<0.05, *p*<0.01 (paired *t*-test).

Table 3. Dietary intake of energy and nutrients per day.

Items	Calculated ¹	Measured						
Energy	7,500 kJ (1,800 kcal)							
Protein	84 g							
Carbohydrate	260 g							
Lipid	24% of energy	Menu						
		No. 1	No. 2	No. 3	No. 4	No. 5	Mean±SD (mg)	Mean±SD (mmol)
Na		2,200	2,300	2,300	2,200	2,200	2,200±100	96±5
(as NaCl)	5.8 g							
K	3.3 g	2,800	2,800	2,200	3,000	2,600	2,700±300	69±8
Ca	830 mg	870	750	710	900	770	800±80	20±2
Mg	280 mg ²	290	270	260	280	320	280±20	12±1
P	1,500 mg	1,490	1,590	1,570	1,720	1,780	1,630±120	53±4
Fe	17 mg	17	37	17	32	33	27±9	0.49±0.17
Zn		28	28	27	26	30	28±2	0.43±0.02
Cu		3.7	4.2	3.9	3.6	7.6	4.6±1.7	0.072±0.027
Mn		3.5	3.7	3.7	3.8	4.7	3.9±0.5	0.070±0.008

¹ After adding our data in minerals for foods not appeared in the tables (semidried oysters and smoked pork liver) (7).

² Based on salt-free ash (SFA) (11).

(1.25–1.5 kp, 50–60 rpm) during the Balance I session. The other three subjects (b, d, f) did the same during the Balance II session. Arm sweat during exercise was collected after cleaning the skin surface with pure water and ion-free treated gauze with ethylene-diamine-tetraacetic acid, diammonium salt (EDTA: Wako Pure Chemical Ind., Ltd., Japan), with the whole arm covered with a long polyethylene bag wrapped with tape. The collected sweat was filtered with a 0.10 µm pore size Teflon filter (Fluoro pore, Sumitomo Electric Ind. Ltd., Japan) and ethanol (for trace analysis, Wako Pure Chemical Ind. Ltd.) to remove any solids.

The diets supplied during the experiment consisted of a 5-d rotating menu and met all normal dietary allowances in Japan (6) as calculated by the food tables (Table 3) (7). To obtain the same quantities of constituents, all foodstuffs were carefully weighed before preparing the dishes (8).

Duplicated and homogenized diets were triplicate sampled, and wet-ashed by hot plates using nitric acid (UGR for trace analysis, Kanto Chemical Co., Ltd., Japan) and hydrogen peroxide (for an atomic absorption analysis, Wako Pure Chemical Ind., Ltd.).

Fecal specimens were collected throughout the experiment and were separated into those originating in the diet during the first and second balance periods according to the ingested coloring marker appearing in the feces. All homogenized fecal samples were treated the same as the diet samples.

In order to obtain an adequate concentration for analysis, all samples (ashed diets, ashed feces, urine and filtered sweats) were diluted with the 0.5 N nitric acid solution. For the determination of Ca, strontium chloride (for atomic absorption analysis, Wako Pure Chemical Ind., Ltd.) was added to the sample at a final concentration of 2,500 ppm.

The minerals (Na, K, Ca, and Mg and dietary iron ([Fe]), zinc [Zn], copper [Cu], and manganese [Mn]) were analyzed by an atomic absorption spectrophotom-

eter (AAS) (Varian AA-5, Australia) using an air-acetylene (for atomic absorption analysis, Toho Acetylene Co., Ltd., Japan) flame.

To determine the Ca levels by this technique, the pH of the standard solution is known to affect the absorption factor (9). Therefore, the height of the burner was adjusted to obtain the same absorption factor in standard solutions having different pHs before measuring the Ca. The dietary phosphorus (P) and urine creatinine were measured by the Fiske-SubbaRow and Folin-Wu methods, respectively (10).

Blood specimens were measured in a commercial laboratory (Biomedical Laboratories, Tokyo, Japan).

For the statistical analysis, the paired *t*-test after ANOVA was used.

RESULTS

Diet

The dietary mineral contents during the 10-d balance sessions are shown in Table 3. The dietary Na, Ca, and Mg were almost the same as that estimated by the Tables (7, 11). However, those of K and P were 80% and 110% of the estimated values, respectively.

24-h urine

The 24-h urine mineral excretion is shown in Fig. 1. The values for Na, on days menu 4 was provided, were significantly higher than those for menu 5 ($p<0.05$, paired *t*-test). Those during the balance sessions were a little below the Na intake.

The values for K throughout the experiment were about 80% of the intake, as is often the case in such experiments (unpublished observation). The values for K on days menu 4 was provided were also significantly higher than those for menu 5 ($p<0.05$, paired *t*-test).

However, the values for Ca throughout the experiment were higher than those in our previous experiments, and also higher than those reported in other literature (12) where less than 200 mg/d of 24-h urine Ca was reported to be normal. The values for Ca on days

Fig. 1. The 24-h urine sodium (Na), calcium (Ca), potassium (K), and magnesium (Mg) excretion from female students ($n=6$) under low Na intake throughout the experiment. Mean daily dietary Na intake was 2.2 g (100 mmol/d) as shown by the horizontal bar in the figure of Na excretion. The Na intake for each menu was 2.2, 2.3, 2.3, 2.2, and 2.0 g/d, respectively, in numerical order. However, the 24-h urine Na on the days menu 4 was fed was significantly higher than that of diet menu 5 in the first three sessions ($p<0.05$, paired t -test). On the other hand, the K intake in each menu was 2.8, 2.8, 2.2, 3.1, and 2.6 g/d, respectively, in numerical order. Note that the urine Ca and Mg excretions were high in this experiment.

menu 4 was provided were also significantly higher than those for menu 5 ($p<0.05$, paired t -test).

The values for Mg throughout the experiment were higher than those measured in our previous experiments except for the Mg supplement study (13), but they were within the reference ranges according to previous reports (8–15 mEq/d [4–7.5 mmol/d] or 3.7–12.9 mEq/d [1.9–6.5 mmol/d]) (14).

Serum minerals and serum or plasma hormones

The minerals in the fasting morning serum are shown in Table 4. The serum Na (S-Na) was significantly lower in the final specimen than that in the other specimens. The serum Mg (S-Mg) was significantly higher in the first specimen than in other specimens. However, no significant changes were observed in either Ca or K.

The plasma renin activity (PRA), angiotensin I (Angio I), and aldosterone (Ald) concentration showed higher values than those in the reference range indicated in the laboratory data throughout the experiment (Table 5).

A slight but significant decrease within the reference ranges in triiodothyronine (T3), thyroxine (T4), and insulin (IRI) were also observed. Among them, changes in T3 and T4 were often observed without low Na

intake in our laboratory. However, significant changes in IRI during the experiment were observed only in this experiment.

Partition urine minerals on days 2, 7, and 12 on days menu 4 was provided

Partition urine (PU) minerals (Na, K, Ca, and Mg) on days 2, 7, and 12, where menu 4 was supplied, are shown in Fig. 2. The mean level of PU-Na on day 2 between 8:30 and 18:30 was higher than that on other

Table 4. The contents of serum minerals on the fasted morning (mean $\pm$ SD).

Items	Day 4	Day 9	Day 14
Na (mmol/L)	140 $\pm$ 2 ^{aaa}	140 $\pm$ 1 ^{bbb}	137 $\pm$ 1 ^{aaa,bbb}
Cl (mmol/L)	105 $\pm$ 2	103 $\pm$ 2	104 $\pm$ 1
K (mmol/L)	4.1 $\pm$ 0.2	4.1 $\pm$ 0.1	4.1 $\pm$ 0.1
Ca (mmol/L)	4.4 $\pm$ 0.1	4.4 $\pm$ 0.2	4.5 $\pm$ 0.1
Mg (mg/dL)	1.9 $\pm$ 0.1 ^{a,b}	1.8 $\pm$ 0.1 ^a	1.7 $\pm$ 0.1 ^b
iP (mg/dL)	3.7 $\pm$ 0.2	3.6 $\pm$ 0.3	3.8 $\pm$ 0.2

^{a,b} $p<0.05$, ^{aaa,bbb} $p<0.001$ between the same letters (paired t -test).

iP, inorganic phosphate.

Table 5. The concentration of serum or plasma hormones on the fasted morning.

Items	Reference range	Day 4	Day 9	Day 14
PRA (ng/mL/h)	0.1–2.0	4.1±1.8 ^a	3.4±1.9	3.0±1.7 ^a
Angio I (pg/mL)	less than 180	194±78	158±49	121±75
Angio II (pg/mL)	less than 50	17±9	22±9 ^a	13±4 ^a
Ald (ng/dL)	less than 18.0	27.1±9.3	19.8±6.1	22.0±3.7
Insulin (μU/mL)	3–18	9±3 ^a	8±2 ^b	7±1 ^{a,b}
T3 (ng/dL)	80–180	132±26 ^a	132±18 ^b	106±12 ^{a,b}
T4 (μg/dL)	5.0–14.0	8.3±1.3 ^a	8.1±1.4 ^b	7.5±1.0 ^{a,b}

^{a,b} $p<0.05$ between the same letters (paired t -test) (mean±SD).
PRA, plasma renin activity; Angio, angiotensin; Ald, aldosteron; T3, triiodothyronine; T4, thyroxin.

Fig. 2. Partition urine minerals (sodium [Na], calcium [Ca], potassium [K], and magnesium [Mg]) on the three days when menu 4 was consumed (mean±sd). Urine was sampled at 06:00 (night), 08:30 (before breakfast; early morning), 12:30 (before lunch; morning), 16:30 (afternoon), 18:30 (before supper), and at 22:00 (before sleep). Values are plotted at the center of each interval (* $p<0.05$, ** $p<0.01$, *** $p<0.001$ paired t -test).

days but the difference was not significant. The levels of PU-Na between 22:00 and 8:30 the next morning were almost the same for all 3 d. Some differences were significant in PU-K, PU-Ca, and PU-Mg.

Sweat minerals.

The concentration of arm sweat minerals (Na, K, Ca, and Mg), and the estimated sweat loss (mineral concentration×body weight loss) during exercise are shown in Table 6. The sweat Na concentration (sw-Na) was not reduced, and sw-Ca and Mg were not as high as

was previously reported (1).

Balance of minerals

The results of the balance of the minerals (Na, K, Ca, and Mg) in this study were calculated as those during the whole balance period (10 d) and the findings are shown in Table 7. The Na balance was slightly negative, while the Ca and Mg balances were obviously negative and the K balance was slightly positive in this experiment.

Table 6. Arm sweat content and estimated dermal loss of minerals.

Na		K	
Concentration (mmol/L)	Dermal output (mmol/trial)	Concentration (mmol/L)	Dermal output (mmol/trial)
Mean±SD (range)	13.2±2.6 (9.4–20.7)	8.0±2.5 (4.3–18.8)	4.5±1.8 (5.4–11.9)
Ca		Mg	
Concentration (mmol/L)	Dermal output (mmol/trial)	Concentration (mmol/L)	Dermal output (mmol/trial)
Mean±SD (range)	0.39±0.11 (0.26–1.01)	0.23±0.04 (0.14–0.31)	0.11±0.13 (0.03–0.41)

Arm sweat was collected during 60 min of moderate exercise under low Na intake of 2.2 g/d (*n* = 30; mean of six subjects in five trials).

Dermal output of minerals (Na, K, Ca and Mg) is calculated by multiplying the concentration by body weight loss.

DISCUSSION

Estimation of Na deficiency in this study

The dietary Na content in this study of 100 mmol/d was half as much as that observed in a normal dietary supply of 200 mmol/d or more for Japanese. However, it is difficult to determine whether or not Na was deficient in this study, because Na deficiency itself has yet to be clearly defined.

It is generally believed that urine Na excretion is restricted by decreasing renal plasma flow and/or increasing tubular reabsorption with the homeostatic action of some mechanisms including the renin-angiotensin-aldosterone system to maintain the plasma Na concentration as well as the plasma volume if the dietary Na is limited. In addition, there are still no known objective symptoms illustrating Na deficiency in humans. However, desirable Na is known to be an objective symptom in some animals that have Na deficiency, and this phenomenon is called a “salt appetite.”

Consequently, in Japan and the United States (15, 16), the Na requirement has been estimated by the minimum Na loss; that is, the sum of inevitable Na loss from the feces, urine, and skin when receiving no dietary Na supply. This Na requirement was evaluated without considering the existence of a Na pool in the body.

In our previous study (1), the lower Na and higher Ca and Mg contents in arm sweat during relatively heavy exercise under a relatively low dietary Na intake of 100 mmol/d suggested both the presence of Na deficiency in that study and the presence of a possible mechanism which compensates for the Na deficiency. The proposed mechanism is considered to act via the elution of Na from the bone, where 50% of the bodily Na is known to be stored. Although it is only one of the minor elements in the bone, Na elution from the bone inevitably exceed that of such major minerals as Ca. In our previous study, the low Na and high Ca and Mg concentrations in arm sweat during relatively heavy

exercise were considered to be the result of increased bone resorption.

This study confirmed existence of the mechanism and illustrated in a balanced technique, that is, the high urine excretions of Ca and Mg, and, the negative balances of Ca and Mg, which did not compensate for any increased intestinal absorption under a sufficient dietary intake of Ca and Mg. These results also suggest an increase in bone resorption, although a direct marker for bone metabolism was not measured in this experiment. Further experimentation is needed to clarify this. In this mechanism, not Ca, but Na thus appears to play the leading role in osteolysis.

In this study, it is further possible to indirectly illustrate the existence of Na deficiency based on the data that the serum Na concentration decreased in the final specimen, while the activity in the renin-angiotensin-aldosterone system, which enhanced the increased renal reabsorption of Na, was higher than the reference ranges throughout the study. Therefore, the Na intake of 100 mmol/d or 2.2 g/d for the subjects, especially in this study, was considered to be deficient, even though the sweat Na level was not low.

The Ca and Mg status in this study

In all subjects, Ca was negatively balanced, although this dietary mineral was not restricted. The dietary allowances of Ca have been assessed to be 600 mg/d in Japan (16), and 1,200 mg/d in the United States (15) for the same age and sex as the subjects of this experiment.

The dietary Ca in this experiment was 800 mg/d, or in a range between the allowances for the Japanese and Americans. It is possible that the dietary Ca was deficient. However, in such a case, the urinary Ca would thus decrease, while the intestinal absorption would increase to compensate for the low dietary Ca level. In this experiment, the urinary Ca was high while the intestinal absorption was not high, and thus the authors concluded that the dietary Ca level was adequate in this experiment, even though the balance of Ca

Table 7. Balance of sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) under low Na intake in six female students for 10 d.

Sodium (Na)						
Sub.	Intake (g/d)	Feces (g/d)	Apparent absorption (%)	Urine (g/d)	Sweat* (g/d)	Balance (g/d)
a	2.21	0.02	99	2.17	0.10	-0.08
c	2.21	0.02	99	2.12	0.07	0.00
e	2.21	0.01	99	2.17	0.10	-0.07
b	2.21	0.01	99	2.19	0.02	-0.01
d	2.21	0.03	99	2.04	0.08	0.06
f	2.21	0.02	99	2.14	0.15	-0.09
Mean		0.02	99	2.14	0.09	-0.03
SD		0.01	0	0.05	0.04	0.06
Potassium (K)						
Sub.	Intake (g/d)	Feces (g/d)	Apparent absorption (%)	Urine (g/d)	Sweat* (g/d)	Balance (g/d)
a	2.71	0.37	86	2.17	0.09	0.07
c	2.71	0.29	89	2.30	0.09	0.04
e	2.71	0.34	87	2.14	0.07	0.16
b	2.71	0.43	84	2.18	0.09	0.01
d	2.71	0.33	88	1.97	0.09	0.33
f	2.71	0.30	89	2.18	0.09	0.14
Mean		0.34	87	2.16	0.09	0.12
SD		0.05	2	0.11	0.01	0.12
Calcium (Ca)						
Sub.	Intake (mg/d)	Feces (mg/d)	Apparent absorption (%)	Urine (mg/d)	Sweat* (mg/d)	Balance (mg/d)
a	802	593	26	337	5	-132
c	802	593	26	312	4	-106
e	802	643	20	285	5	-130
b	802	623	22	261	6	-88
d	802	694	13	171	5	-68
f	802	643	20	175	5	-21
Mean		631	21	275	5	-91
SD		38	5	70	1	42
Magnesium (Mg)						
Sub.	Intake (mg/d)	Feces (mg/d)	Apparent absorption (%)	Urine (mg/d)	Sweat* (mg/d)	Balance (mg/d)
a	283	181	36	114	3	-15
c	283	174	39	120	0	-11
e	283	196	31	107	0	-20
b	283	204	28	86	1	-8
d	283	189	33	107	0	-13
f	283	174	39	116	0	-7
Mean		186	34	108	1	-12
SD		12	4	12	1	5

* Sweat loss of minerals were mean of 10 d with and without exercise.

was negative.

The dietary minimum Mg requirement was considered to be 160 mg/d for the Japanese in our laboratory (13, 17). The dietary allowance of Mg is 280 mg/d in the United States (15) and 250 mg/d in Japan for the subjects (18).

Based on the above data, the dietary Mg was thus not considered to be deficient. However, it is still possible that there was a deficiency of Mg. In such a case, the urinary Mg would decrease and intestinal absorption would increase to compensate for the low dietary Mg level.

The urinary Mg was high while the intestinal absorption was moderate, and thus the dietary Mg was not found to be deficient in this experiment, even though both the serum Mg decreased during the experiment, and the balance of Mg was also negative.

Dietary Na intake and urine Ca and Na

On the other hand, it has been reported that an excess salt intake increases urine Ca excretion, and that the urine Na and Ca levels also showed a positive correlation (19), which thus suggested that sodium plays a completely different role in the Ca metabolism in this study.

In another previous balance study of ours, where salt was supplied on two levels in a crossover manner, the urine Ca excretion level during a session of higher salt intake was higher than that during lower salt intake (unpublished observation). As a result, the authors do not deny the presence of a possible mechanism in which an excess sodium intake causes an increase in urine Ca excretion by increasing the renal plasma flow.

In this experiment, the urine creatinine (the data is not shown in this paper) and the mineral (Na, K, Ca, and Mg) excretions on the days that menu 4 was provided were significantly higher than those when menu 5 was fed. Therefore, the results of this experiment support, in part, the positive correlation between urine Na and Ca levels. This correlation between Na and Ca may be related to the dietary K intake following the increased urine K excretion in this experiment, as shown in Fig. 1. The decreased serum insulin during the experiment thus supported the hypothesis that the dietary K level was sufficient throughout the experiment. However, the correlation between the urine Na and Ca levels does not directly support the hypothesis that a high sodium intake leads to a negative balance of Ca, which thereafter leads to the development of osteoporosis (19) because the intestinal absorption of Ca is also regulated.

Uresis of Ca and Mg

Independent of the Na intake, various risk factors for chronic degenerative diseases such as physical and mental stress (9, 20), overeating (20, 21), and anaerobic exercise (2) also increase urine Mg and Ca levels. Under these circumstances, urine Ca and Mg are generally excreted in an isomolar ratio (same molar volume). To keep the plasma Ca constant, the Ca in the bone is eluted since the bone is the sole physiological Ca pool in the body. However, it is difficult to determine exactly

which organ contributes to maintaining the plasma Mg at a constant level. If the plasma Mg was maintained not by soft tissue, but by the bone, extensive bone Ca would thus be eluted with the Mg from the bone because of the high Ca/Mg molar ratio in the bone (at least more than five) (22). As a result, regarding the uresis of Ca and Mg, at least some Mg must be delivered from the soft tissue in order to help protect the supply of the bone minerals, especially Ca (20).

In this experiment, the urine Ca level was much higher than that observed in other experiments (9, 13). It was also higher than that of Mg regarding the molar ratio. Even though the reason why the urine Ca was higher than that of Mg in this experiment could not be clarified, Mg in soft tissue does not appear to strongly contribute to the urine Mg level, in which Na is not physiologically stored.

Theory of osteoporosis without low dietary Ca intake

The results of this experiment as well as those of our previous studies (1–3, 9, 20, 21) strongly suggest that some mechanisms to remove bone minerals without low dietary Ca intake must exist, although no detailed mechanism has yet been identified. Those mechanisms may be activated when any of the minerals stored in the bone (Na, Ca, Mg, P, and zinc [Zn]) become deficient in the body.

Due to such mechanisms, osteoporosis will inevitably progress even if the dietary Ca level is sufficient.

Ca metabolism in this experiment

It is possible that the high urine Ca excretion and negative Ca balance seen in this experiment could also be the result of some interaction between the Ca regulating hormones and the vitamins. However, these factors were not measured in this study.

Some circumstances, such as a high protein intake (23) or exposure to low gravity during space travel (24–26), are already known to cause a negative Ca balance in spite of a sufficient dietary Ca intake.

The mechanism to illustrate such a negative Ca balance with sufficient Ca intake is usually not directly involved in the metabolism of minerals. However, our findings suggest that a negative Ca balance appears to be the result of a deficiency of other minerals stored in the bone (1, 2, 9, 20, 21).

Although the negative Ca and Mg balances observed in this study have yet to be proven to be the result of Na deficiency, it should be kept in mind, when evaluating the sodium requirement that bone sodium is eluted when Na is deficient both in the body and when an excessive degree of bone resorption is induced.

CONCLUSION

The balances of minerals (Na, K, Ca, and Mg) were measured under a relatively low Na intake (100 mmol/d or 2.2 g/d) with an adequate intake of Ca (20 mmol/d or 800 mg/d) and Mg (12 mmol/d or 280 mg/d) in six female students for 10 d. The plasma renin activity (PRA) and aldosterone level were above the reference ranges throughout the experiment, which suggests that Na deficiency existed in the subjects. However, the uri-

nary Na excretion was about the same as that which had been ingested in the given diet. On the other hand, the urine Ca and Mg were high, while the apparent absorption of Ca and Mg were moderate ($21 \pm 5\%$, $34 \pm 4\%$, respectively), which resulted in a negative balance of these two elements. It is thus believed that the stored Na in the bone is eluted to compensate for the low dietary Na intake, while excess Ca and Mg also inevitably flow into the blood stream during bone resorption, which both inhibits intestinal absorption and accelerates urine excretion of these minerals.

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