Salt Restriction Affects the Excretions of Minerals (Na, K, Ca, Mg, P and Zn) in the Second Voided Fasting Early Morning Urine

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Summary The plasma concentrations of mineral (sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), and zinc (Zn)) are kept within narrow ranges to maintain homeostasis; hence, it is difficult to use them as indicators of nutritional status. We selected the excretion of these minerals in the second voided fasting early morning urine (EMU) as potential indicators of nutritional status. We previously reported that Na restriction caused a negative balance of Ca and Mg. Therefore, Na restriction can cause changes in EMU-minerals. This study aimed to examine the relationship between dietary Na restriction and urinary mineral excretion. The study lasted for 21 d, including 16 d of balance period and 3 d of recovery period. The participants (11 healthy young women) were divided into the Na restriction group (n=5) (NaCl: 6 g/d) and control group (n=6) (NaCl: 12 g/d). The Na restriction group changed to the control diet (NaCl: 12 g/d) during only the recovery period. The EMU-Na, Ca, Mg, P and Zn in the Na restriction group significantly decreased compared with that of the control group. The EMU-Na, K, Ca, Mg, and Zn in the group with NaCl intake of 6 g/d significantly decreased compared with that of the group with NaCl intake of 12 g/d (in the Na restriction group). We conclude that the decrease in excretion of Na, Ca, Mg and Zn in the EMU can lead to Na restriction. This result can serve as basis when considering EMU as an indicator of mineral status.

Key Words salt restriction, sodium (Na) intake, urinary minerals, second voided fasting early morning urine (EMU), excretions of minerals

We previously reported that the urinary calcium (Ca) and magnesium (Mg) excretions are increased by intake of large quantity of energy (1, 2), and by exposure to mental or physical stress (3, 4), which are known to be risk factors for chronic degenerative diseases such as diabetes and hypertension, and are decreased by light exercise (5), one of the known preventive factors against chronic diseases. In addition, the intake of these minerals is important when considering the etiology of these diseases. We also conducted metabolic balance studies of minerals in humans to establish the requirement of these minerals (6–9). The next approach is to find several indexes to diagnose the nutritional status (excess or deficit) of these minerals (10, 11). Plasma levels of minerals are one of the possible indexes of human health, but they are kept within narrow ranges when the deficits are moderate; therefore, they are not considered suitable for this purpose. Hence, we focused on urinary minerals as potential indexes for mineral status. We considered urinary mineral excretions in the 24-h urine and the second voided fasting early morning urine (EMU) as indicators of mineral status. Spot urine is not suitable for this purpose, because urinary mineral excretion is known to have diurnal variation, high in daytime and low at night, and affected by diet.

In this study, we chose the EMU, the urine obtained at the farthest distance from the immediate diet. Under an insufficient Mg diet (160 mg/d), EMU-Mg decreased significantly (12), indicating that EMU is a good marker. By contrast, Sodium (Na) restriction reduced the absorption of Cu and Mg and caused the negative balances of these minerals (6). Therefore, Na restriction may cause changes in EMU-Ca and Mg. Following the above hypothesis, we measured the EMU-minerals (Na, potassium (K), Ca, Mg, phosphorus (P), and zinc (Zn)) of the Na restriction group (NaCl: 6 g/d) and compared them with those of the control group (NaCl: 12 g/d).

SUBJECTS AND METHODS

We examined the data of human metabolic balance study conducted in 2004. This study was conducted in
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accordance with the rules of the Helsinki Declaration and approved by the Ethics Committee of the National Institute of Health and Nutrition (application number 04003; date of approval: July 18, 2004). The participants were composed of 11 healthy Japanese women, aged 19–23 y (Table 1). The duration of this study was 21 d, including 16 d of balance period, with 3 d recovery period after the balance period (Fig. 1). The participants stayed on a ward at the National Institute of Health and Nutrition throughout the experiment and were divided into two groups: Na restriction group (n=5, NaCl intake: 6 g/d) and control group (n=6, NaCl intake: 12 g/d).

Registered dieticians, in accordance with the Dietary Reference Intakes for Japan (13), prepared each meal, based on the Standard Tables of Food Composition in Japan (14). They prepared a 4-d rotating menu for Na restriction diet (NaCl: 6 g/d) under the condition that the meal can be supplied uniformly to the participants and meal samples (15). The control group ingested the same Na restricted diet but with 2 g of salt in each meal (6 g/d) as the control diet (NaCl: 12 g/d) every day throughout the study. The Na restriction group ingested a Na restricted diet during the balance period (NaCl: 6 g/d), but they changed to the control diet (NaCl: 12 g/d) during the recovery period. The dietary energy, protein, lipid, carbohydrate, and minerals supplied during the study are shown in Table 2. The EMU is the urine collected after initial voiding upon arising, but before breakfast. Fasting blood samples were collected on experiment days 1, 5, 9, 13 and 17 (Fig. 1).

Five minerals (Na, K, Ca, Mg, and Zn) were analyzed using an atomic absorption spectrophotometer (AAS, Varian AA-5, Australia) after the mixture was diluted to an appropriate concentration with 0.5 mol/L of nitric acid followed by wet ashing with nitric acid and hydrogen peroxide if necessary. P was measured by a colorimeter (molybdenum blue method). This human metabolic balance study produced various results other than those reported in the EMU-mineral study, which were described elsewhere (15, 16). In addition, other experimental conditions were described in the previous studies (8, 16, 17). The minerals in the blood were analyzed by the BML Laboratories Inc. (BML, Inc.; SRL, Inc.). The relationship between Na intake and EMU-minerals (Na, K, Ca, Mg, P and Zn) was analyzed as the parameters per body weight (BW) (12). BW is the participant’s average fasting morning BW throughout the balance period. The Na intake in the previous day before the EMU collection was used in the analysis. The data of EMU-minerals during the balance periods were used to compare between Na restriction group (n=5) and the control group (n=6). The data of EMU-minerals in the final balance period (step 4: NaCl 6 g/d) and recovery period (NaCl 12 g/d) using the same dietary menu except the meal (6 g/d) as the control diet (NaCl: 12 g/d) every day throughout the study.

Table 1. Characteristics of subjects (11 females).

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na restriction (n=5) (NaCl intake 6 g/d)</td>
<td>20.6±0.5</td>
<td>160.8±6.8</td>
</tr>
<tr>
<td>Control (n=6) (NaCl intake 12 g/d)</td>
<td>20.8±1.3</td>
<td>159.4±5.9</td>
</tr>
</tbody>
</table>

Significant differences between the items were not observed.

**Table 1.** Characteristics of subjects (11 females).

**Experiment Design**

| Experimental day | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|------------------|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|
| Step             |   |   |   |   |   |   |   |   |   |   | (Step1) | (Step2) | (Step3) | (Step4) | Recovery period |
| Menu number      | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| NaCl intake of Na restriction group | 6 g/d | 12 g/d |
| NaCl intake of control group | 12 g/d |
| Samples          |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Meal             | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| EMU*2            | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| Fasting blood    | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| Data of intergroup comparison | Na restriction (NaCl intake 6 g/d) vs. Control (NaCl intake 12 g/d) |
| Data of intragroup comparison | (within Na restriction group) |

Fig. 1. Experiment protocol. This metabolic balance study consisted of two phases: the balance period (step 1, step 2, step 3, and step 4), and the recovery period. The participants consumed a diet consisting of a 4-d-rotating menu throughout the study. The 24-h urine was collected from 8:30 am to 8:30 am the next day as one day. The second voided fasting early morning urine (EMU) was obtained from 6:00 am (the first voiding in the morning: night urine) to 8:30 am (before breakfast), collected separately from the other urine. In the morning of the days 1, 5, 9, 13, and 17, fasting blood samples were collected. The NaCl intake of the Na restriction group was 6 g/d during the balance period, but it increased to 12 g/d during the recovery period. The EMU data during the balance period were used to compare between the Na restriction group and the control group, as the intergroup comparison. In the Na restriction group, the EMU data in step 4 and the recovery period were used for the intragroup comparison.

*[^1]{1,6} ad lib
*[^2]{1,6} Collected separately from 6:00 (Waking up) to 8:30 (Breakfast) (2.5mlU)
intake of Na were used for intracomparison of EMU-minerals in the Na restriction group (Fig. 1). Single correlations between Na intake and EMU-minerals were obtained using the data of all participants (n=11). We utilized the blood samples for statistical test without the first collection (1 d of the experiment), because first collected blood samples were unaffected by the Na intake of this study. We used IBM SPSS Statistics version 22, 24 for statistical analysis.

RESULTS

Relationships between serum mineral concentrations (serum minerals) and Na intake (intergroup comparisons during the balance period)

The serum mineral concentrations of the Na restriction and control group during the balance period are shown in Table 3. Serum K, Mg and P of the Na restriction group were higher than those of the control group. The correlation between Na intake and serum Na, Ca and Zn were not significant. Relationships between EMU-minerals and Na intake (intergroup comparisons during the balance period)

EMU-minerals in the Na restriction and control group during the balance period are shown in Table 4. All minerals measured except K were significantly different between the Na restriction group and the control group. EMU-Na, P, Ca, Mg and Zn in the Na restriction group were lower than those of the control group at the intergroup comparison. Furthermore, the correlation between Na intake and EMU-minerals were calculated using all samples in both groups (n=175). As a result, the correlation was positively significant between Na
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However, the correlation between Na intake and EMU-K was not significant (Fig. 2).

Relationship between Na intake and EMU-minerals in the Na restriction group (intragroup comparison)

EMU-minerals at the recovery period (NaCl: 12 g/d; successive 3 d and the mean of the 3 d) were compared with those at corresponding balance period of step 4 (NaCl: 6 g/d). The mean EMU-Na, K, Ca, Mg, P and Zn at the recovery period were significantly higher, but the EMU-P was significantly lower than those at the corresponding balance period of step 4 (Table 5).

Table 5. EMU-minerals at the intragroup comparison in the Na restriction group (mean for the 3 d) (n=5).

<table>
<thead>
<tr>
<th></th>
<th>NaCl 6 g/d (Step 4)</th>
<th>NaCl 12 g/d (Recovery)</th>
<th>Unit</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMU-Na</td>
<td>0.78±0.34</td>
<td>2.12±0.82</td>
<td>μmol/kg BW/min</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EMU-K</td>
<td>0.46±0.18</td>
<td>0.75±0.20</td>
<td>μmol/kg BW/min</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EMU-P</td>
<td>176±37</td>
<td>152±40</td>
<td>nmol/kg BW/min</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EMU-Ca</td>
<td>19.0±8.8</td>
<td>27.0±13.6</td>
<td>nmol/kg BW/min</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EMU-Mg</td>
<td>34.4±8.2</td>
<td>40.9±8.8</td>
<td>nmol/kg BW/min</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EMU-Zn</td>
<td>49.1±9.3</td>
<td>59.5±9.1</td>
<td>pmol/kg BW/min</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean±SD. Paired-t test

![Graphs showing relationships between Na intake and EMU-minerals in the balance period (intergroup comparison).](image-url)
DISCUSSION

Relationships between serum minerals and Na intake

As homeostasis maintains the plasma mineral concentrations within a very constant range, it is difficult to determine the mineral intake based on blood mineral concentrations. However, in our present results, serum K, Mg and P concentrations were significantly higher in the Na restricted group than in the control group, suggesting that Na intake in the Na restriction group was significantly lower than those in the control group (intergroup comparison). Hence, it should be noted that Na affects serum minerals, but if it is fluctuating within the reference value, the conventional blood tests can hardly reflect or reveal the deficiency and/or excess of mineral, and the health status thereof.

Relationships between the Na intake and the EMU-minerals (intergroup comparison)

Although all participants ingested the same amounts of minerals other than Na, the EMU-Na, Ca, Mg, P, and Zn in the Na restriction group was significantly lower than those in the control group (intergroup comparison). This finding suggested that Na intake in the Na restriction group (NaCl: 6 g/d) is actually insufficient (8, 20) and several of the Na metabolic functions are performed in the kidneys as well as in the extrarenal systems. Na metabolism regulation is mainly carried out in the kidney and is mediated by the renin-angiotensin-aldosterone system and natriuretic peptide system. Aldosterone acts on the Na pump in the collecting duct of the kidney to promote Na reabsorption (21). These systems are also involved in the Na resorption from the bone (22), and this action affects not only Na but also Ca, Mg, P, and Zn, for all of them are stored in the bone (bone minerals). Moreover, we have considered that since K is not reserved in the bone, the EMU-K does not show any significant change. Furthermore, significant positive correlations were found between the EMU-Na, Ca, Mg, P, and Zn and Na intake (Fig. 2).

Relationships between Na intake and EMU-minerals in the Na restriction group (intragroup comparison)

The mean EMU-Na, K, Ca, Mg and Zn at the recovery period were significantly higher, but EMU-P was significantly lower than those at the corresponding balance period of step 4 (Table 5). The differences between intergroup and intragroup comparisons are the changes in EMU-K and EMU-P. Increase in EMU-K at the recovery period (NaCl: 12 g/d) may be explained as transit effects of increasing K absorption accompanied with the increase in intake and absorption of Na (20). Decrease in EMU-P during the recovery period was possibly caused by several factors. Decrease in urine P may increase Ca absorption. Some of Ca may be stored in the bone along with P, and urine P may be decreased to compensate the increase in bone P. Increased absorption of Na, Ca, and Mg (bone mineral accumulation) may support this explanation (20).

Mechanism by which Na restriction decreases the EMU-Ca and Mg

We have reported that there are significant positive correlations between Na intake and Ca and Mg balance (6). As mentioned in the introduction, when intake of salt is low (NaCl: 6 g/d), the absorption of Ca and Mg from the intestinal tract is suppressed, and the same bone minerals (Ca and Mg) are released from the bone with Na (20). This study showed that the absorption of Ca and Mg from the intestinal tract was suppressed in participants with low Na intake, and that reabsorption in the kidney was improved in order to maintain blood Ca and Mg concentrations. As with Ca and Mg, P and Zn, constituting bones are considered to be the same mechanism as those. However, we could not clarify the mechanisms of these results in this study. Hence, future research should clarify the relationship between EMU-minerals and blood hormone concentrations as well as the influence of Na on other mineral metabolisms.

CONCLUSION

The authors conclude that Na restriction as low as 6 g/d of NaCl, is one of the factors that can cause changes in the amounts of the EMU-minerals (Na, K, Ca, Mg, P, and Zn). Moreover, Na restriction causes changes in EMU-minerals. Dietary Na may be one of the key factors to control mineral (Na, K, Ca, Mg, P, and Zn) metabolism.

Acknowledgments

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