Minerals in Plasma and Urine and Gustatory Function Tests for Salt in a Group of Healthy Young Men

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Summary Detection threshold for salt (NaCl) and discrimination between two levels of NaCl concentration (0.6 and 0.7%) in foods, and their relation to some selected biochemical parameters in plasma and urine (Zn, Na, K, Mg, Ca, Se for plasma and urine, Cu and retinol binding protein for plasma) were investigated in 15 healthy male college students. No subject failed to discriminate the NaCl concentrations in more than 50% of the tests. The rate of correct discrimination (RCD) was not associated with plasma Zn (P-Zn), plasma retinol binding protein (P-RBP), urinary potassium (U-K) or urinary sodium (U-Na), which significantly correlated with RCD in our previous study, while the detection threshold was significantly correlated with urinary Ca-Mg ratio (U-Ca/Mg), urinary Ca (U-Ca), U-Na, and urinary Mg. In the stepwise multiple regression analysis, U-Ca/Mg, plasma Ca, plasma Na, and RCD were selected as significant independent variables. These indicate that the status of minerals such as Na, Ca, and Mg is related to the gustatory function. One possible explanation for the discrepancy between the present and previous results is the elevated P-Zn and P-RBP levels in the present subjects.

Key Words discrimination, salt concentrations, detection threshold, taste of salt, minerals, plasma, urine, sodium, potassium, calcium, magnesium, zinc, copper, selenium, retinol binding protein

Plasma or serum zinc (Zn) concentration is considered to be the best single available laboratory test for Zn deficiency, even though the standard reference value is set in consideration of its variable character including diurnal variation (1). Taste
acuity testing has been expected to serve as a sensitive indicator of mild Zn deficiency (2); thus it is necessary to clarify the relationship between plasma Zn concentrations and taste acuity in order to establish criteria for evaluation of Zn nutrition. Aspects of gustatory sense other than taste acuity, e.g., taste intensity perception, have been claimed to be more reproducibly related to Zn nutrition than is the taste acuity threshold (2), but there have been few studies dealing with this possibility.

In our previous study, 17 out of 64 college-aged women had lowered ability to discriminate between two levels (0.6 and 0.7%) of salt concentration in water and food (3); the discriminability was positively associated with the plasma Zn and retinol levels and negatively with urinary levels of sodium (Na) and potassium (K), but the detection threshold for salt concentration did not show any significant relation to these biochemical parameters. The average plasma Zn concentration in the group with poor ability to discriminate was less than 0.7 µg/ml, the lowest value of the normal range identified by Hambidge et al. (1), while that in the group with good ability to discriminate was about 0.74 µg/ml, and plasma Zn concentrations weakly and significantly correlated with plasma retinol binding protein (RBP) but not with plasma retinol (3). This result indicated that the gustatory disfunction of this type in this group was related to not only mild Zn deficiency but also other patho-physiological mechanisms such as mild deficiency of vitamin A, coincidental with or accompanied by mild Zn deficiency, and elevated consumption of salt. In view of the fact that the discrimination between salt concentrations is related to many factors, the specificity of the discrimination test as an indicator of mild Zn deficiency has to be examined for subjects under various states of Zn nutrition using a large number of examination items. We studied a group of young healthy men, and did not find a significant association of plasma or urinary Zn with gustatory functions, but found a possible association of calcium (Ca) and/or magnesium (Mg), in addition to Na, which was already recognized as a significant factor in the previous report (3).

SUBJECTS AND METHODS

Subjects. Fifteen male students, who were studying physical education in college and gave their informed consent to this study, were examined in August 1984 for their detection threshold for salt concentration, discriminability of salt concentrations, and some selected biochemical parameters. Ten out of 15 were undergraduate students and members of a wrestling club; they were engaged in fairly heavy exercise most days. The remaining 5 were graduate students engaged in scientific studies on physical education, and their physical activity was moderate.

Gustatory function tests. The detection threshold for the taste of salt (NaCl) which was dissolved in distilled water and discrimination between two levels of NaCl concentrations (0.6 and 0.7%) in drinking water, soups and cooked food (boiled rice and potato) were tested as in the previous report (3) on the afternoons of
August 21 (for detection threshold and discrimination for boiled rice), 23 and 24 (discrimination testing for two kinds of samples on each day).

**Blood and urine collection.** The blood was venipunctured from the cubital vein into heparinized tubes between 7 and 8 a.m. after an overnight fast on August 22. The urine was collected for 24 h beginning at 8 a.m. August 22.

**Chemical analysis.** Hemoglobin concentration (Hb) in blood was determined by the cyan-methemoglobin method. Zinc, copper (Cu), Ca, Mg, Na, and K concentrations in plasma were measured by flame atomic absorption spectrometry (Nippon Jarell Ash, Model AA-845) after appropriate dilution with distilled water. In the case of Ca, lanthanum chloride was added at the final concentration of 1% to avoid the interferences of phosphate. Plasma selenium (Se) concentrations were measured by Watkinson’s fluorometric method (4). To ensure accuracy of determination, a reference serum (National Inst. Environ. Studies of Japan, NIES No. 4) was measured simultaneously at each time of measurement. Retinol binding protein (RBP) in plasma was estimated by radial immunodiffusion (5) using Partigen plates (Hoechst).

For 24-h collected urine samples, creatinine was colorimetrically measured using Jaffe’s reaction (6). On the basis of the creatinine value, it was suspected that some subjects’ urine samples were not complete collections over the 24-h period; therefore, urinary elemental concentrations were described only as related to creatinine. Elements measured for urine were Na, K, Ca, Mg, Zn, and Se: All but Se were measured by flame atomic absorption spectrometry as in the case of plasma. Selenium was measured by the same method as that for plasma.

**Anthropometric measurement.** Stature and weight were measured just prior to this experiment, on August 21, and the body mass index (BMI, weight, kg/the square of the stature, m²) was calculated (mean ± SD of age, stature, weight and BMI are shown in Table 1).

**Statistical analysis.** From the results of calculation of skewness and kurtosis, deviation from the normal distribution was substantially suspected for the Na-to-K ratio in urine, and the logarithmic conversion was applied to this for statistical analysis. Correlation analysis and stepwise multiple regression analysis were applied to reveal the interrelationship between the parameters examined (correlation) and to select the variables which may be related to gustatory functions (multiple regression) (7).

| Table 1. Age, stature, weight, and body mass index of subjects (n = 15). |
|-----------------|-----------------|
| **Mean** | **Standard deviation** |
| Age (years) | 20.5 | 3.0 |
| Stature (cm) | 169.3 | 6.1 |
| Weight (kg) | 56.3 | 7.8 |
| BMI | 23.1 | 1.8 |
RESULTS

**Discrimination of NaCl concentrations and detection thresholds**

In five tests of discrimination, rates of correct discrimination (RCD) on a group basis varied from 50% for boiled rice to 100% for miso soup (Table 2). This result—i.e., the easiest to discriminate was miso soup and the most difficult was boiled rice among the five test matrices—was quite similar to that in the previous study (3). Four students gave correct discrimination for all the tests; 8 students gave 4 correct discriminations, and the remaining 3 students 3 correct discriminations. What differed from the result in the previous study was that there were no subjects with frequent failure (over 50%) in repeated discrimination tests.

In 8 of 14 students tested, the detection threshold was at the level of 6 mmol/liter; in one it was at the level of 12 mmol/liter; and in the remaining 5, at the level of 30 mmol/liter.

**Biochemical parameters**

No anemic subjects were found (Table 3). Though data are not shown, hepatic and renal functions tested were all normal. No significant elevation of serum cholesterol and triglycerides levels was found.

As is shown in Table 3, the average plasma Zn (P-Zn) level was 0.81 μg/ml with a range from 0.71 to 0.95 μg/ml, being significantly higher than that (0.73 μg/ml, range: 0.53–0.94 μg/ml) in the subjects of the previous study (p < 0.01 by t-test). The gender of the subjects and the different time of blood sampling must be discussed in this regard.

Among the items, whose average levels can be compared with the previous data on female students, plasma Cu (P-Cu), urinary Ca (U-Ca), and urinary Mg (U-Mg) did not show significant differences. Higher levels in the present group were noted for P-Zn as mentioned, urinary Zn (U-Zn), hemoglobin, and plasma RBP; in contrast, lower levels were found in urinary sodium (U-Na) and potassium (U-K) (Table 3). When compared with the reference normal range in clinical chemistry (8), the present plasma Na, Ca, and Mg averages exceeded the upper level of the normal range.

**Table 2. Discrimination between two levels of salt concentration (0.6 and 0.7%) (n=14 or 15).**

| Test sample                | No. of correct discriminators (%) |
|----------------------------|-----------------------------------|
| Boiled rice                | 7 (50)                            |
| Boiled potato              | 11 (73.3)                         |
| *Miso* soup                | 15 (100)                          |
| Japanese-style consommé soup | 14 (93.3)                       |
| Water solution             | 13 (86.7)                         |

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Table 3. Gustatory function tests, blood and urine biochemical parameters.

| Item                        | n  | Mean | SD  | Range  | Previous study* |
|-----------------------------|----|------|-----|--------|-----------------|
| RCD * (%)                   | 15 | 81.3 | 14.1| 60     | 83.2 (17–100)   |
| Threshold (mmol/liter)      | 14 | 15.0 | 11.7| 6      | 9.8 (6–30)      |
| Hemoglobin (g/dl)           | 15 | 15.2 | 0.6 | 14.0   | 13.2 (10.5–14.8)**c |
| Plasma Zn (µg/ml)           | 14 | 0.81 | 0.07| 0.71   | 0.73 (0.53–0.94)** |
| Plasma Cu (µg/ml)           | 14 | 0.79 | 0.10| 0.64   | 0.84 (0.61–1.17) |
| Plasma Na (mg/ml)           | 13 | 3.73 | 0.14| 3.37   | 3.91            |
| Plasma K (mg/ml)            | 13 | 0.16 | 0.01| 0.14   | 0.19            |
| P-Na/K (mol/mol)d           | 13 | 40.0 | 3.2 | 34.1   | 46.0            |
| Plasma Ca (µg/ml)           | 13 | 106  | 6.0 | 98     | 116             |
| Plasma Mg (µg/ml)           | 13 | 23.3 | 2.2 | 18.7   | 25.8            |
| P-Ca/Mg (mol/mol)           | 13 | 2.79 | 0.23| 2.46   | 3.17            |
| Plasma Se (µg/ml)           | 14 | 0.11 | 0.012| 0.09 | 0.13            |
| Plasma RBP (mg/dl)          | 13 | 5.8  | 1.2 | 3.6    | 4.7 (2.9–6.6)*  |
| Urinary Zn (µg/g cr)        | 15 | 420  | 77  | 233    | 197 (26–844)*   |
| Urinary Na (g/g cr)         | 15 | 2.3  | 0.7 | 1.1    | 3.6 (1.5–8.2)*  |
| Urinary K (g/g cr)          | 15 | 1.3  | 0.5 | 0.6    | 1.8 (0.5–3.9)** |
| U-Na/K (mol/mol)d           | 15 | 3.9  | 3.6 | 1.8    | 16.4            |
| Urinary Ca (mg/g cr)        | 15 | 113  | 64  | 31     | 122 (29–444)    |
| Urinary Mg (mg/g cr)        | 15 | 77   | 22  | 40     | 75 (32–181)     |
| U-Ca/Mg (mol/mol)           | 15 | 0.83 | 0.33| 0.35   | 1.36            |
| Urinary Se (µg/g cr)        | 15 | 35.6 | 11.8| 19.3   | 58.7            |

*Data on 63 or 64 female college students (see Ref. 3 for details). bRCD, rate of correct discrimination of salt concentrations (see the text for details). c*p<0.05; **p<0.01 by t-test in comparison of the present mean with the previous value. dP- and U- are plasma and urine.

Results of correlational analysis

Rates of correct discrimination did not significantly correlate with any of the biochemical parameters (the largest correlation coefficient was found for the Na-to-K ratio in plasma (P-Na/K) \(r = -0.455, p = 0.118\)). The detection threshold for salt was significantly correlated with some parameters such as urinary Ca-Mg ratio \(r = -0.754, p = 0.002, U-Ca (r = -0.728, p = 0.003), U-Na (r = -0.592, p = 0.026),\) and \(U-Mg (r = -0.541, p = 0.046), U-Zn (r = -0.493, p = 0.073)\) followed these, though not at a significant level.

In the correlation matrix, age is one of the factors which have been correlated with many variables. The subjects studied were from two groups, as noted: a younger group which exercised actively and a slightly older group with moderate physical activity. Therefore, the significant correlation with age may have resulted from different levels of physical activity.
Table 4. Correlation matrix between age, hemoglobin concentration; P-, plasma; U-, urinary; RBP, retinol binding protein in plasma; THLD, detection threshold for salt concentration; RCD, rate of correct detection.

| Hb | P-Zn | P-Cu | P-Se | P-Ca | P-Mg | P-Ca/Mg | P-Na | P-K | P-Na/K | RBP |
|----|------|------|------|------|------|---------|------|-----|--------|-----|
|    |      |      |      |      |      |         |      |     |        |     |
|    |      |      |      |      |      |         |      |     |        |     |
|    |      |      |      |      |      |         |      |     |        |     |
|    | .636 |      |      |      |      | -.603   | -.629 |     |        | .568 |
|    |      | .542 |      |      |      |         |      |     |        |     |
|    |      |      | .600 |      |      |         |      |     |        |     |
|    |      |      |      | .548 |      |         |      |     |        |     |
|    |      |      |      |      | .541 | .562    |      |     |        |     |
|    |      |      |      |      |      |         |      |     |        |     |
|    |      |      |      |      |      | -.824   | .693  | .666 |        |     |

Hb, hemoglobin concentration; P-, plasma; U-, urinary; RBP, retinol binding protein in plasma; THLD, detection threshold for salt concentration; RCD, rate of correct detection.

Table 5. Results of stepwise regression analysis.

| Dependent variable | Independent variables | Standardized partial regression coefficient | t-Value |
|--------------------|-----------------------|---------------------------------------------|---------|
| RCD none           |                                     |                                             |         |
| THLD R=0.953       | U-Ca/Mg                | -1.044                                      | -8.369***|
|                    | P-Ca                   | -0.701                                      | -5.003**|
|                    | RCD                    | -0.437                                      | -3.692**|
|                    | P-Na                   | 0.520                                       | 3.580** |

*** p<0.001, ** p<0.01. R, multiple correlation coefficient. Abbreviations, see Table 4.

Plasma Zn levels have significant correlations with P-Se (negatively) and P-Mg (positively). Urinary Zn was significantly correlated with Hb, P-Se, P-Mg, and P-K. Urinary level of Na was significantly correlated with U-Ca/Mg.
biochemical parameters, and gustatory functions.

| U-Zn | U-Se | U-Ca | U-Mg | U-Ca/Mg | U-Na | U-K | U-Na/K | THLD | RCD |
|------|------|------|------|---------|------|-----|--------|------|-----|
|      |      |      |      |         | .552 |     | .602   |      | Age |
| -.579|      |      |      |         |      |     |        |      | Hb  |
|      |      |      |      |         |      |     |        |      | P-Zn|
| -.711|      |      |      |         | .563 |     |        |      | P-Cu|
|      | -.545|      |      |         |      |     |        |      | P-Se|
| .584 |      |      |      |         |      |     |        |      | P-Ca|
|      |      |      |      |         |      |     |        |      | P-Mg|
|      |      |      |      |         |      |     |        |      | P-Ca/Mg|
| .583 |      |      |      |         | .617 |    | .735   |      | P-Na|
|      |      |      |      |         |      |     |        |      | P-K |
|      |      |      |      |         |      |     |        |      | P-Na/K|
|      |      |      |      |         |      |     |        |      | RBP |
|      |      |      |      |         |      |     |        |      | U-Zn|
| .852 |     | .910 | .554 |         | -.728 |   | .640   |      | U-Se|
| .606 |     |     |      |         |      |     |        |      | U-K |
| .621 |     |     |      |         |      |     |        |      | U-Na/K|
|      |     |     |      |         |      |     |        |      | THLD |

Results of stepwise regression analysis

In the stepwise regression analysis, no significant independent variable explaining RCD was extracted, while 4 variables—U-Ca/Mg, P-Ca, RCD, and P-Na—were significant in explaining the detection threshold for salt concentration (Table 5). The first three variables had negative coefficients; the last, a positive coefficient.

DISCUSSION

In contrast with the previous results (3), neither P-Zn, P-RBP, U-K, or U-Na have been associated with RCD; and moreover, no other variables showed significant association with RCD. The absence of anyone who frequently failed to discriminate salt concentrations and the higher levels of P-Zn and P-RBP and the lower level of U-Na among the present subjects may have caused the lack of significant association. However, the present blood sampling was conducted early in the morning after an overnight fast, while it was done in the afternoon in the previous study. Diurnal variation in plasma or serum Zn levels has been noticed (9–11), and P-Zn levels in the afternoon sample were about 10–20% less than...
in the early morning sample in our own study of subjects on an ordinary meal schedule (12). Therefore, the present P-Zn level after rectification of diurnal variation may be comparable with the previous level in female students on the average, but there remain a few female students with remarkably low values. For differences owing to gender, the results reported are inconsistent (13–17), and we cannot discuss the comparability of P-Zn data in this respect. Plasma RBP did not show a significant diurnal variation in our observation (12). The difference between the present and previous subjects is, thus, considerable.

In contrast to RCD, the detection threshold for salt concentration was related to U-Ca/Mg, P-Ca, P-Na, and RCD in the regression analysis. On the basis of the close interrelations of U-Na, U-Ca, and U-Ca/Mg, the primary entrance of U-Ca/Mg into the multiple regression equation must be interpreted to represent the relevance of these elements to the gustatory functions. In this sense, the entrance of P-Ca and P-Na, both of which showed elevated levels and correlated with P-Mg, into the equation is quite supportive of the present thinking. As a pathological condition which influenced the metabolism of Ca and the gustatory functions, pseudohypoparathyroidism was described (18). Hypercalcemia in adults may occur as a result of hyperparathyroidism or excessive intake or hypersensitivity to vitamin D (19). The present subjects were slightly hypercalcemic. From concurrent elevation of P-Na and P-Mg with P-Ca, low levels of U-Na and U-K and hard exercise in 10 out of 15 subjects, we can suspect a change in water and mineral metabolism, although no direct evidence can be cited.

In the previous study (3), RCD seemed to be independent of the detection threshold, but in the present multiple regression equation, RCD entered as a significant variable to explain the threshold. This suggests a need to study more carefully the relation of RCD to the detection threshold.

Apart from relations of biochemical parameters to gustatory functions, the significant correlations of P-Se with P-Zn (negatively), U-Zn (negatively) and U-Na (positively), and of U-Se with P-Ca (negatively), U-Ca/Mg (positively) and U-K (positively), elicit interest in another way. Interaction of selenium with zinc in vitro and in vivo has been recognized by many researchers (20–23), but the interaction between two metals in the human body has not been studied. The present result may be a first indication of possible interaction.

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