Diurnal Variation of Plasma Minerals and Trace Elements in a Group of Japanese Male Adults

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Summary When the nutritional status of minerals and essential trace elements is assessed by their levels in plasma, intra-individual variation in the measured values, particularly diurnal variation, must be considered. In this paper, concentrations of nine elements (Na, Mg, P, K, Ca, Fe, Cu, Zn, and Se) in plasma collected 7 times in a 24-h period from 10 healthy Japanese adults were measured with hemoglobin and plasma concentrations of proteins (total protein, albumin, retinol binding protein (RBP), ceruloplasmin, and transferrin), total cholesterol, and cortisol. Then the pattern of diurnal variation in, and the interrelationships among, these parameters were clarified in subjects who consumed an ordinary meal. Significant diurnal variation examined by two-way analysis of variance (variations due to subject and sampling time) was found for Zn, RBP, and cortisol. Plasma Zn level was higher in the forenoon samples than in those taken in the afternoon. The pattern of diurnal variation found for cortisol was similar to, but slightly different from, that for Zn. The correlation coefficient between Zn and cortisol was significant (r = 0.555) using the standardized values for individuals, and in the result of multiple regression analysis, cortisol level was selected as the significant explanatory factor for Zn variation; however, the sampling time was the most significant

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factor. For other elements besides Zn, no significant diurnal variation was found. Moreover, no meaningful factors were selected for variations of those elements in the result of multiple regression analysis. These results indicate that, in assessing Zn nutriture with its plasma levels, it is necessary to unify the sampling time.

**Key Words** diurnal variation, minerals, trace elements, plasma, nutritional assessment

Plasma or serum levels of minerals and essential trace elements have been used to assess their nutritional status for populations as well as individuals (1). In these cases, it is necessary to know, in advance, the various causes of intra-individual variation in the measured values. One of the most important factors may be diurnal variation, fluctuation due to the time of day at which sampling is done.

The fluctuation of plasma (or serum) concentrations of elements such as magnesium (Mg), potassium (K), calcium (Ca), iron (Fe), copper (Cu), and zinc (Zn) was investigated (2-15). Some of the investigations suggested that these element concentrations in plasma did not fluctuate randomly during the day, but had significant diurnal variation over a 24-h period. However, the pattern of diurnal variation differed between the various elements; even for the same element, it differed according to the experimental design, i.e., the interval of the sampling time, the meal time and the length of the fasting condition, and the kind of meal (ordinary meal or liquid experimental diets). When significant diurnal variation was found for the plasma element levels, the regulating factors have been suggested to be the plasma carrying protein such as albumin for Ca (3) and ceruloplasmin for Cu (2), adrenocortical steroid hormones such as cortisol for Mg and Ca (5), parathyroid hormone for Ca (3), and meal per se for Zn (9, 11).

In this paper, blood samples were collected 7 times over a 24-h period, from 7:30 on the first day of the experiment to 7:30 on the following day, from 10 healthy Japanese adult men; concentrations of nine elements (sodium (Na), Mg, phosphorus (P), K, Ca, Fe, Cu, Zn, and selenium (Se)) in plasma were measured with hemoglobin level (HB) and plasma concentrations of proteins (total protein (TPRO), albumin (ALB), retinol binding protein (RBP), ceruloplasmin (CP), and transferrin (TF)), total cholesterol (CHOL), and cortisol (CORT). Then we obtained the pattern of diurnal variation in, and the interrelationships among, these parameters in subjects who consumed an ordinary meal.

**SUBJECTS AND METHODS**

The subjects of this study were 10 healthy male volunteers (3 staff members and 7 students of our Department), 23 to 39 (mean 28.3) years of age. Mean body weight was 66.8 ± 8.2 kg. They participated after listening to an explanation of its objectives and methodologies and giving their informed consent. The study
procedure was approved by the Committee on Human Studies at the Faculty of Medicine, University of Tokyo.

Seven blood samples were collected from each subject. The initial samples were collected at 7:30 after an overnight fast (from 20:00), and then five samples were collected at 2.5-h intervals to 20:00 (10:00, 12:30, 15:00, 17:30, and 20:00). After a second overnight fast, the last samples were collected at 7:30 the following day. During the study period, except for water, all subjects were restricted to three meals (at 8:00, 13:00, and 18:00) prepared by us. Daily energy and protein intake provided by these meals was 2,360 kcal and 95.0 g, respectively. All of the subjects' time in the daytime was spent in sedentary work.

At each sampling, about 5 ml of blood was drawn from the cubital vein by a disposable plastic syringe and transferred into a polypropylene tube with heparin as an anti-coagulant. Hemoglobin concentration was measured by the cyanmethemoglobin method. The plasma sample was separated by centrifugation and stored in a freezer (−20°C) until analysis. Eight element concentrations (Na, Mg, P, K, Ca, Fe, Cu, and Zn) were measured by Inductively Coupled Plasma Atomic Emission Spectrometry after digestion with HNO₃ in a Teflon-lined, high pressure decomposition vessel (San-ai Kagaku Co.), and Se was measured by Watkinson’s fluorometric method (16) after digestion with HNO₃ and HClO₄. To ensure the accuracy of the determination, a reference serum (National Inst. Environ. Studies of Japan, NIES, No. 4) was measured simultaneously. Plasma TPRO, ALB, and CHOL concentrations were measured using an auto-analyzer (Abbott Bichromatic Analyzer, ABA 200), and RBP, CP, and TF concentrations were measured with the radial immunodiffusion method. Plasma CORT concentration was measured with radioimmunoassay.

Statistical analyses—paired t-test, two-way analysis of variance (ANOVA) with Duncan’s multiple range test for the comparison of mean level, correlation analysis, and multiple regression analysis—were conducted using the SAS computer program package (17). In correlation analysis, the standardized value (Z score: difference from mean divided by standard deviation) calculated for each subject was used to minimize the effect of inter-individual variation. Multiple regression analysis was applied, using standardized values to clarify the factors determining the diurnal variation of plasma element levels. In addition to HB, TPRO, ALB, RBP, CP, TF, CHOL, and CORT, the factors of MEAL (sampling at 30 min before meal=0, sampling at 2 h after meal=1) and TIME (sampling time, from 7:30=1 to 20:00=6) were used as independent variables.

**RESULTS**

Table 1 shows mean and standard deviation of each item by sampling time. The difference between mean levels of the samples collected under fasting conditions (7:30 samples) on the first day of the experiment and the following day was examined by paired t-test. As shown in the right-hand column, mean levels of Fe, Zn, and
| Item                        | 7:30      | 10:00     | 12:30     | 15:00     | 17:30     | 20:00     | 7:30     |
|---------------------------|-----------|-----------|-----------|-----------|-----------|-----------|----------|
| Sodium (μg/ml)            | 3076(218) | 3256(225) | 3169(188) | 3161(255) | 3192(153) | 3112(197) | 3159(169) |
| Magnesium (μg/ml)         | 21.4(1.9) | 21.2(2.0) | 22.4(2.5) | 22.1(2.1) | 22.2(2.8) | 20.7(2.8) | 22.3(2.7) |
| Phosphorus (μg/ml)        | 122(20)   | 114(14)   | 120(15)   | 117(17)   | 121(15)   | 116(11)   | 114(13)   |
| Potassium (μg/ml)         | 148(23)   | 147(29)   | 150(21)   | 145(19)   | 152(25)   | 146(24)   | 145(19)   |
| Calcium (μg/ml)           | 94.0(8.9) | 94.2(3.5) | 98.2(11.1)| 97.6(11.2)| 96.2(13.2)| 90.2(11.0)| 96.7(11.2)|
| Iron (μg/ml)              | 1.17(0.35)| 1.35(0.37)| 1.28(0.35)| 1.47(0.41)| 1.39(0.36)| 1.41(0.56)| 1.86(0.42)| p<0.01    |
| Copper (μg/ml)            | 0.80(0.24)| 0.82(0.23)| 0.82(0.20)| 0.78(0.18)| 0.80(0.18)| 0.77(0.16)| 0.76(0.14)| ns        |
| Zinc (μg/ml)              | 0.74(0.13)| 0.73(0.14)| 0.70(0.09)| 0.64(0.10)| 0.62(0.08)| 0.61(0.10)| 0.82(0.13)| p<0.05    |
| Selenium (μg/ml)          | 0.132(0.020)| 0.135(0.024)| 0.138(0.020)| 0.132(0.022)| 0.135(0.019)| 0.132(0.020)| 0.133(0.016)| ns        |
| Hemoglobin (g/100ml)      | 15.2(1.1) | 15.4(1.2) | 15.3(1.0) | 15.2(1.1) | 15.4(1.0) | 15.3(1.2) | 15.6(1.1) | p<0.01    |
| Total Protein (g/100ml)   | 7.0(0.6)  | 7.4(0.7)  | 7.4(0.5)  | 7.4(0.6)  | 7.4(0.5)  | 7.5(0.6)  | 7.2(0.6)  | ns        |
| Albumin (g/100ml)         | 4.3(0.4)  | 4.3(0.2)  | 4.4(0.2)  | 4.4(0.2)  | 4.4(0.3)  | 4.4(0.3)  | 4.3(0.2)  | ns        |
| Retinol Binding Protein (mg/100ml) | 5.2(0.6)  | 5.3(0.6)  | 5.5(0.7)  | 5.3(0.6)  | 5.3(0.6)  | 5.2(0.5)  | 5.3(0.4)  | ns        |
| Ceruloplasmin (mg/100ml)  | 23.2(4.4) | 23.5(4.1) | 24.0(4.1) | 23.2(4.0) | 23.8(3.9) | 23.3(3.7) | 23.4(3.6) | ns        |
| Transferrin (mg/100ml)    | 296(36)   | 293(30)   | 305(40)   | 297(38)   | 304(31)   | 299(32)   | 297(32)   | ns        |
| Total Cholesterol (mg/100ml)| 209(26)   | 211(27)   | 216(32)   | 223(29)   | 218(33)   | 216(35)   | 215(26)   | ns        |
| Cortisol (μg/100ml)       | 10.0(3.4) | 7.8(2.2)  | 9.3(3.9)  | 6.1(1.5)  | 6.6(3.4)  | 6.1(2.1)  | 13.1(4.7) | ns        |

*Comparison of mean levels of the two fasting samples (time 7:30) on the first and second day of experiment. ns, not significant.
Table 2. Inter- and intra-individual variation (CV) and $F$-values of the result of two-way ANOVA (variations due to subject and sampling time).a

|                  | Inter-individual variation CV(%) | Intra-individual variation CV(%) | Two-Way ANOVA (F-values) variation due to subject | Two-Way ANOVA (F-values) variation due to sampling time |
|------------------|----------------------------------|----------------------------------|-----------------------------------------------|----------------------------------------------------------|
| Sodium           | 6.5 (1.1)                        | 4.2 (1.3)                        | 10.6 ***                                       | 2.3                                                       |
| Magnesium        | 10.9 (1.9)                       | 7.3 (3.1)                        | 8.3 ***                                        | 1.6                                                       |
| Phosphorus       | 13.0 (2.3)                       | 8.4 (5.3)                        | 6.3 ***                                        | 0.8                                                       |
| Potassium        | 16.0 (2.3)                       | 8.4 (4.9)                        | 13.8 ***                                       | 0.4                                                       |
| Calcium          | 11.4 (1.5)                       | 7.9 (4.1)                        | 6.7 ***                                        | 1.4                                                       |
| Iron             | 29.7 (4.9)                       | 25.2 (10.2)                      | 2.8 *                                          | 0.9                                                       |
| Copper           | 24.7 (3.4)                       | 6.2 (2.9)                        | 58.6 ***                                       | 1.2                                                       |
| Zinc             | 16.3 (2.5)                       | 10.8 (4.3)                       | 16.5 ***                                       | 9.4 ***                                                   |
| Selenium         | 15.6 (1.4)                       | 5.2 (1.2)                        | 48.8 ***                                       | 1.1                                                       |
| Hemoglobin       | 7.3 (0.5)                        | 1.9 (0.5)                        | 77.5 ***                                       | 0.7                                                       |
| Total Protein    | 7.8 (0.9)                        | 5.4 (1.3)                        | 7.9 ***                                        | 1.7                                                       |
| Albumin          | 5.9 (1.9)                        | 4.7 (1.6)                        | 4.5 ***                                        | 0.9                                                       |
| Retinol Binding  | 11.4 (1.3)                       | 3.2 (2.2)                        | 54.2 ***                                       | 3.1 *                                                     |
| Protein          |                                  |                                  |                                               |                                                           |
| Ceruloplasmin    | 17.2 (1.0)                       | 3.7 (1.4)                        | 118.2 ***                                      | 1.3                                                       |
| Transferrin      | 12.1 (1.3)                       | 3.4 (1.4)                        | 66.7 ***                                       | 1.8                                                       |
| Total Cholesterol| 14.1 (1.5)                       | 7.2 (3.1)                        | 13.5 ***                                       | 0.8                                                       |
| Cortisol         | 35.9 (10.0)                      | 39.2 (8.7)                       | 2.0                                            | 3.9 **                                                    |

a The data for 7:30 of the second day were excluded from analysis ($n=60$). *** $p<0.001$, ** $p<0.01$, and * $p<0.05$, respectively.

HB on the second day were significantly higher than those on the first day.

Table 2 shows inter- and intra-individual variation (variation due to subject in each sampling time and that due to sampling time in each subject) expressed by the mean of coefficient of variation (CV, %), and the results of two-way ANOVA expressed by the $F$-values for the variations due to subject and sampling time. In these analyses, the data collected at 7:30 on the second day were excluded ($n=60$). Inter-individual variation ranged from 5.9% in ALB to 35.9% in CORT. For intra-individual variation, most items except Fe, Zn, and CORT showed CV levels of less than 10%. In the results of two-way ANOVA, variation due to subject was significant for most items except for CORT, while that due to sampling time was significant only for the three items Zn, RBP, and CORT.

For these three items which showed significant variation with the sampling time, the patterns of diurnal variation are shown in Figs. 1 to 3. In these figures, the value at 7:30 on the second day is also plotted.

Mean plasma Zn level was highest in the fasting sample (7:30), and it decreased gradually with time to the lowest level at 20:00, which was 82.5% of the level at 7:30 (Fig. 1). In the result of two-way ANOVA with Duncan's multiple range test, the difference among the mean level of each sampling time was significant between the levels from 7:30 to 12:30 and those from 15:00 to 20:00. When the individual levels were compared for the first day of the experiment, the highest level was found...
Fig. 1. Pattern of diurnal variation of plasma zinc concentration. a, b: Mean with the same character does not significantly differ by Duncan’s multiple range test in the result of two-way ANOVA.

Fig. 2. Pattern of diurnal variation of plasma retinol binding protein (RBP) concentration. a, b: Mean with the same character does not significantly differ by Duncan’s multiple range test in the result of two-way ANOVA.

at 7:30 for 5 subjects, at 10:00 for 4 subjects, and at 12:30 for one subject; the lowest level was found at 17:30 for 3 subjects and at 20:00 for 7 subjects. The ratios of the lowest to the highest levels ranged from 64.8 to 88.8% (mean of 76.7%), and the correlation coefficient between these two levels was 0.782 (p<0.01).

The variation in plasma RBP was significant, but the difference in the mean level at each sampling time was not so great (Fig. 2). Plasma CORT levels at 7:30 and 12:30 were higher than those at 15:00 to 20:00 (Fig. 3).

Table 3 shows the correlation coefficient matrix calculated using all the data (n=70), and Table 4 shows that using standardized values (only significant correlation coefficients are shown). Both with and without standardization values, there are high correlations (p<0.001) between the pairs Mg and P, Mg and Ca, P
Fig. 3. Pattern of diurnal variation of plasma cortisol concentration. a, b: Mean with the same character does not significantly differ by Duncan’s multiple range test in the result of two-way ANOVA.

and Ca, and TPRO and ALB. In some pairs, such as Cu and CP, the significance disappeared in the standardized value, though there was high correlation in the value without standardization ($r = 0.806$). The correlation coefficient between Zn and CORT, on the other hand, increased from 0.395 to 0.555 with standardization.

The result of multiple regression analysis using the values with standardization is shown in Table 5, with $F$-value, the square of adjusted multiple correlation coefficient ($R^2$), and standardized partial regression coefficient ($\beta$) of each independent variable selected as significant variables. Significant $F$-values were found for Mg, P, Ca, and Zn; however, the $R^2$ value for Mg, P, and Ca were as low as 0.174, 0.295, and 0.149, respectively. $R^2$ was the highest for Zn (0.703), and HB, CP, CORT, and TIME were selected as significant explanatory factors.

**DISCUSSION**

Among the plasma elements examined in this study, only Zn showed the significant variation with sampling time. Dietary Zn intake provided in this
### Table 3. Correlation coefficient matrix

|          | Na   | Mg   | P    | K    | Ca   | Fe   | Cu   | Zn   |
|----------|------|------|------|------|------|------|------|------|
| Sodium   |      |      |      |      |      |      |      | .418*** |
| Magnesium| —    |      |      |      |      |      |      |      |
| Phosphorus| —    |      |      |      |      |      |      | .475*** |
| Potassium| —    |      |      |      |      |      |      | .573*** |
| Calcium  | —    |      |      |      |      |      |      | .389*** |
| Iron     | —    |      |      |      |      |      |      | .608*** |
| Copper   | —    |      |      |      |      |      | .337*  |      |
| Zinc     | —    |      |      |      |      |      |      | .471*** |

***, ***, * p<0.001, p<0.01, and p<0.05, respectively.

### Table 4. Correlation coefficient matrix calculated

|          | Na   | Mg   | P    | K    | Ca   | Fe   | Cu   | Zn   |
|----------|------|------|------|------|------|------|------|------|
| Sodium   | —    |      | .274* |      |      |      |      | .505*** |
| Magnesium| —    |      | .276* |      |      |      |      |      |
| Phosphorus| —    |      | .724*** |      |      |      |      |      |
| Potassium| —    |      | .392*** |      |      |      |      |      |
| Calcium  | —    |      | .394*** |      |      |      |      |      |
| Iron     | —    |      | .961*** |      |      |      |      |      |
| Copper   | —    |      | .411*** |      |      |      |      |      |
| Zinc     | —    |      | .728*** |      |      |      |      |      |
| Selenium | —    |      |      |      |      |      |      |      |
| Hemoglobin| —    |      |      |      |      |      |      |      |
| Total Protein | —    |      |      |      |      |      |      |      |
| Albumin  | —    |      |      |      |      |      |      |      |
| Retinol Binding Protein | —    |      |      |      |      |      |      |      |
| Ceruloplasmin | —    |      |      |      |      |      |      |      |
| Transferrin | —    |      |      |      |      |      |      |      |
| Total Cholesterol | —    |      |      |      |      |      |      |      |
| Cortisol | —    |      |      |      |      |      |      |      |

***, ***, * p<0.001, p<0.01, and p<0.05, respectively.

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calculated using all the data ($n = 70$).

| Se  | HB  | TPRO | ALB | RBP | CP  | TF  | CHOL | CORT |
|-----|-----|------|-----|-----|-----|-----|------|------|
| -.330** | -.269* | .285* | .304* | .241* | .427*** | -.544*** | -.364** | Na  |
| -.286* | .307** | -.292* | -.246* | -.376** | -.243* | -.318** | Mg  |
| .496*** | .516*** | .580*** | .330** | .395*** | Zn  |
| -- | .285* | .419*** | .304* | .723*** | .267* | .327** | Se  |
| -- | -.256* | -- | .751*** | .466*** | .250* | .432*** | HB  |
| -- | -- | .610*** | .488*** | .573*** | TPB  |
| -- | -- | .756*** | .583*** | -- | CP  |
| -- | -- | .459*** | -- | -- | CHOL |
| -- | -- | -- | -- | -- | CORT |

using the standardized values ($n = 70$).

| Se  | HB  | TPRO | ALB | RBP | CP  | TF  | CHOL | CORT |
|-----|-----|------|-----|-----|-----|-----|------|------|
| .437*** | .247* | .247* | .316** | .307** | .307** | -.423*** | Na  |
| .366** | .469*** | .366** | .291* | .291* | .291* | Mg  |
| .409*** | .544*** | .364** | .245*** | .274* | .262* | .555*** | P   |
| -- | -- | .823*** | .368** | .430*** | .242* | .709*** | K   |
| -- | -- | -- | .742*** | .742*** | .742*** | TPB  |
| -- | -- | -- | -- | -- | -- | ALB  |
| -- | -- | -- | -- | -- | -- | -- | RBP |
| -- | -- | -- | -- | -- | -- | -- | CP  |
| -- | -- | -- | -- | -- | -- | -- | TF  |
| -- | -- | -- | -- | -- | -- | -- | CHOL|
| -- | -- | -- | -- | -- | -- | -- | CORT|

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Table 5. The result of multiple regression analysis using the values with standardization.

| Dependent Variables | F-value | $R^2$ | $\beta$ of Independent Variables |
|---------------------|---------|-------|----------------------------------|
|                     |         |       | HB | TPRO | ALB | RBP | CP | TF | CHOL | CORT | MEAL | TIME |
| Na                  | 1.16    | .022  |     |      |     |     |    |    |      |      |      |      |
| Mg                  | 2.46*   | .174  |     |      |     |     |    |    |      |      |      |      |
| P                   | 3.89*** | .295  |     |      |     |     |    |    |      |      |      |      |
| K                   | 1.51    | .069  |     |      |     |     |    |    |      |      |      |      |
| Ca                  | 2.21*   | .149  |     |      |     |     |    |    |      |      |      |      |
| Fe                  | 0.65    | .000  |     |      |     |     |    |    |      |      |      |      |
| Cu                  | 0.80    | .000  |     |      |     |     |    |    |      |      |      |      |
| Zn                  | 17.37***| .703  |     |      |     |     |    |    |      |      |      |      |
| Se                  | 1.96    | .123  |     |      |     |     |    |    |      |      |      |      |

$\beta$, standardized partial regression coefficient; $R^2$, square of adjusted multiple correlation coefficient; $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.
experiment was 8.0 mg/day, which was within the range of ordinary intake levels of Japanese (18). When subjects were permitted to follow their ordinary meal habits, some reports (4, 5, 9, 12, 14) showed a pattern of diurnal variation in plasma (or serum) Zn concentration identical to that found in this study. Namely, plasma Zn showing the highest level in the fasting condition in the morning decreased during the day to about 70–80% of the fasting value in the late afternoon or evening, and rose again during the night. From the contrastive result that serum Zn level was unchanged in the continuous fasting condition during the morning without breakfast, it has been suggested that food intake is the dominant factor in the diurnal variations in serum Zn level (9). The finding by Richards et al. (11) that food intake decreased plasma Zn concentration supported this suggestion. Moser and Gunderson (19) reported that plasma Zn concentration increased when 25 mg Zn as zinc oxide was administered without breakfast. In contrast, when breakfast containing 2.9 mg of Zn was provided prior to the supplement, plasma Zn decreased for at least 3 h after supplementation (19). From these results, they suggested that the fall in plasma Zn after food ingestion may have been due to the movement of Zn from the extracellular to the intracellular compartment, or due to the promoted Zn excretion in the feces or urine (11, 19).

In this study, however, significant decrease of plasma Zn was found only between before- and after-lunch samplings; in the result of multiple regression analysis, the factor of MEAL was not selected as the explanatory factor for plasma Zn variation. McBean and Halsted (20) reported no difference between the fasting and the postprandial plasma Zn level. In addition, the different pattern of diurnal variation in plasma Zn from that mentioned above was reported in the investigation with a liquid experimental diet provided at 8:00, 16:00, and 0:00 (2), or with reversed day-night schedules (5). These results indicated that food intake could not adequately explain the diurnal variation in plasma Zn, and suggested that it was important to consider the experimental design, i.e., the difference between ordinary meals and liquid experimental diets or different time schedules for the experiment including sleeping and meal times.

Significant diurnal variation was found for plasma cortisol level, which was identical to the pattern reported by others (5, 21). This pattern of diurnal variation of CORT (Fig. 3), with levels in the forenoon samples higher than those in the afternoon samples, was similar to that of Zn (Fig. 1). However, a difference was found at the 10:00 sampling, at which the CORT level showed a slight decrease. The correlation coefficient between Zn and CORT with the standardized value was 0.555 (p < 0.001, Table 4). In addition, the result of multiple regression analysis indicated that CORT remained as significant explanatory factor after controlling for the effect of TIME (Table 5). These results did not negate the possibility that plasma Zn level was related to CORT level directly, or that the same mechanism might regulate both Zn and CORT in plasma. However, the experiment with reversed day-night schedules (5) showed a different pattern of diurnal variation for Zn and CORT. In any case, the factor of TIME had the largest β value among
the independent variables used in the multiple regression analysis in this study.

Other elements in plasma showed no significant diurnal variation in this study. Some reports suggested that the diurnal variations of some elements were regulated by plasma carrying protein. For example, significant diurnal variations in serum were shown to be parallel between Ca and ALB(3) and Cu and CP(2). In our study, among the plasma proteins, only RBP showed significant (though not so conspicuous) diurnal variation (Table 2, Fig. 2). In the correlation analysis with standardized values, no strong relationships were found between plasma element and protein levels (the highest value was 0.425 for Se and RBP) (Table 4). Significant correlation between Cu and CP ($r = 0.806$) for the total 70 samples disappeared when values standardized for each individual were used. Moreover, in the multiple regression analysis, plasma protein levels were not selected as the factor explaining the plasma element levels, except for CP for Zn, which showed a negative $\beta$ value.

Plasma Mg levels correlated highly with Ca levels; correlation coefficient was 0.802 for the total 70 samples (Table 3), and it increased to 0.951 with standardized values (Table 4). This result suggested that the levels of these two elements in plasma fluctuated synchronously, and the same mechanism might regulate these two elements levels in plasma, although neither Mg nor Ca shows any significant variation with the sampling time. Lanuza and Marotta (5) showed significant diurnal variation in both plasma Mg and Ca levels, which were directly related, and the 24-h patterns of these two elements were correlated with that of cortisol. In this study, however, in the results of multiple regression analysis, meaningful parameters were not found for plasma Mg and Ca levels (the $R^2$ value was 0.174 for Mg and 0.149 for Ca, Table 5).

It was not clear why in this study only Zn in plasma showed significant diurnal variation, although the variation of plasma Zn was thought to be related to plasma CORT level. The result indicated that it is important to consider the sampling time when the nutritional status of Zn is to be evaluated together with plasma level. Hambidge et al. (22) suggested that the lower limit of the normal plasma Zn level was 0.68 $\mu$g/ml, but they did not specify the sampling time. A significant correlation was found between the highest levels in the forenoon samples and the lowest levels in the afternoon samples in this study; however, a difference as large as 25% indicated that the criteria level for assessing Zn nutriture should be changed in conjunction with sampling time. Conclusively, when the nutritional status of Zn is compared among individuals or populations, it is necessary to unify at least the sampling time.

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