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Evaluation of the Expression of Urinary Selenium Level as ng Se/mg Creatinine and the Use of Single-Void Urine as a Sample for Urinary Selenium Determination

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Interest in the biological significance of selenium has been confined to its toxic effects on animals in the early stage of study, while its essentiality has been noted in recent years. Of almost all trace elements, Se belongs to a "highly toxic" group (BOWEN 1966) and its salts are actually the most toxic on a molar basis. Moreover, it is characteristic of Se that there are only narrow safety margins among the deficient, nutrient and toxic doses (VENUGOPAL & LUCKEY 1978). Therefore, adequate means of assessing Se status are now needed for human health and disease and the present methods used need more evaluation to assure their specificity and reliability (BURK 1976). Of body fluid samples used to assess human Se status, urine seems to be one of the most common (VALENTINE et al.1978). Twenty-four hour urine collections, not spot checks of urine, should be used as they are not subject to error from meal and dilution effects (BURK 1976). 24-h urinary excretion, however, had the disadvantage of difficulty of collection (THOMSON & ROBINSON 1980). The indications hitherto used for urinary Se level were largely Se concentration, ng Se/ml urine. The purpose of this study is to show the advantage of 24-h urinary Se concentration expressed in terms of creatinine concentration, ng Se/mg creatinine, over ng Se/ml urine as indications of Se level in view of nonsusceptibility to variation and dilution effects, and to present the validity of estimation of urinary Se levels by using single void urines as substitutes for 24-h urine samples.

EXPERIMENTAL

24-h urine samples were collected from three healthy subjects once a month during seven months from July to January. All the individual void samples of a 24-h urine sample set were obtained from eight healthy subjects. Urinary volume, and Se and creatinine (abbreviated as CT) concentrations were measured. The determination of Se was made by fluorimetric method with 2,3-diaminonaphthalene (WATKINSON 1966) and that of CT by Folin-Wu method (BOSNES & TAUSSKY 1945).

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RESULTS

Selenium levels expressed as Se(ng/ml), Se(μg) and Se(ng/mg CT) in 24-h urine samples were compared with respect to variation and dilution effects. The results are summarized in Tables 1 and 2 and Figs. 1 and 2. Table 1 gives the mean, standard deviation and coefficient of variation of Se levels. The variables, Se(ng/mg CT) and Se(μg), were found to tend to have lower degree of variation than Se(ng/ml) as is evident from coefficient of variation. The effect of urinary volume on Se levels is given in Table 2 and Fig.1. Urinary Se(ng/ml)

TABLE 1. Selenium levels[a] in 24-h urine during seven months.

Subject	Se (ng/ml)	Se (ng/mg CT[b])	Se (μg)	No. of samples
No.1	46 + 24 (52)[c]	33 + 15 (45)	63 + 26 (41)	7
No.2	61 + 19 (31)	36 + 13 (36)	40 + 13 (33)	7
No.3	35 + 18 (51)	36 + 6 (17)	33 + 5 (15)	7
Mean	47 + 20 (45)	35 + 11 (33)	45 + 15 (30)	

[a]Value is mean ± standard deviation(SD). [b]Creatinine.
[c]Value in parentheses is coefficient of variation, (SD/mean) x 100.

TABLE 2. Correlation coefficient for Se levels in 24-h urine during seven months.

Subject	Se(ng/ml)-Volume(ml)	Se(ng/mg CT[a])-Volume(ml)	Se(μg)-Volume(ml)	Se(ng/ml)-CT(mg/ml)	N[b]
No.1	-0.83	-0.63	-0.74	0.51	7
No.2	-0.44	-0.05	0.18	0.38	7
No.3	-0.88	-0.14	-0.34	0.95	7
Mean	-0.72	-0.27	-0.30	0.61	
|Mean|[c]	0.72	0.27	0.42	0.61	

[a]Creatinine. [b]Number of samples.
[c]Mean of absolute coefficient of correlation.

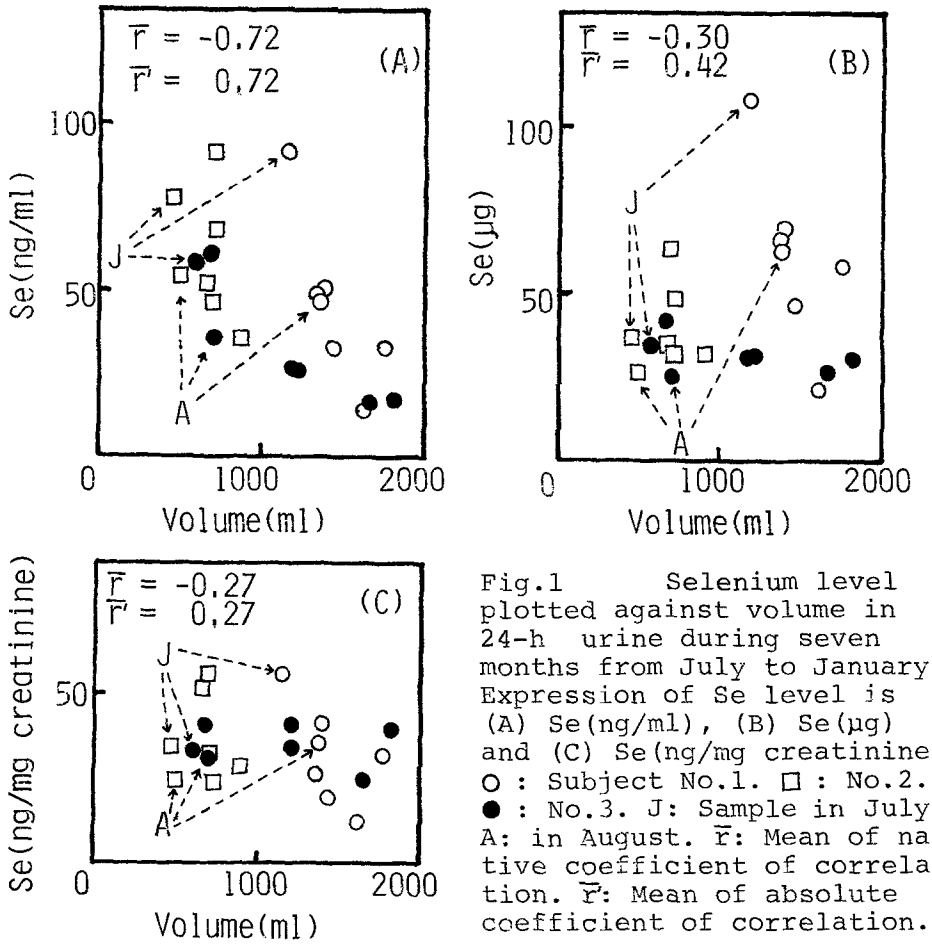


Fig.1 Selenium level plotted against volume in 24-h urine during seven months from July to January. Expression of Se level is (A) Se(ng/ml), (B) Se(μg) and (C) Se(ng/mg creatinine) ○ : Subject No.1. □ : No.2. ● : No.3. J: Sample in July. A: in August. $\bar{r}$: Mean of native coefficient of correlation. $\bar{r}'$: Mean of absolute coefficient of correlation.

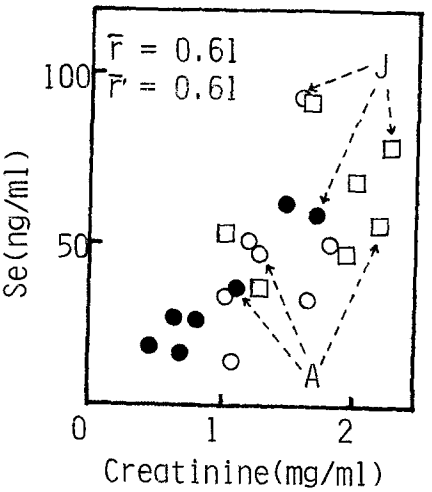


Fig.2. Selenium concentration plotted against creatinine concentration in 24-h urine during seven months from July to January. See Fig.1 for symbols.

was inclined to increase with decreasing urine volume, and particularly was a high level in the summer season most likely because of small volume of 24-h urine. Both the means of native and absolute coefficients of correlation of urine volume with Se(ng/mg CT) were lower than those with Se(μg) and especially with Se(ng/ml). This means that Se(ng/ml) has the disadvantage of great dependence on urine volume. Fig.2 gives plot of Se vs. CT concentrations in daily urine. This good correlation is perhaps responsible for both the low degree of variation and the slight dependence on urine volume which were found in Se(ng/mg CT) of 24-h urine. These results suggest that 24-h urinary Se(ng/mg CT) may be advantageous as a monitor of Se status in view of nonsusceptibility to variation and dilution effects in comparison with Se(ng/ml) and Se(μg).

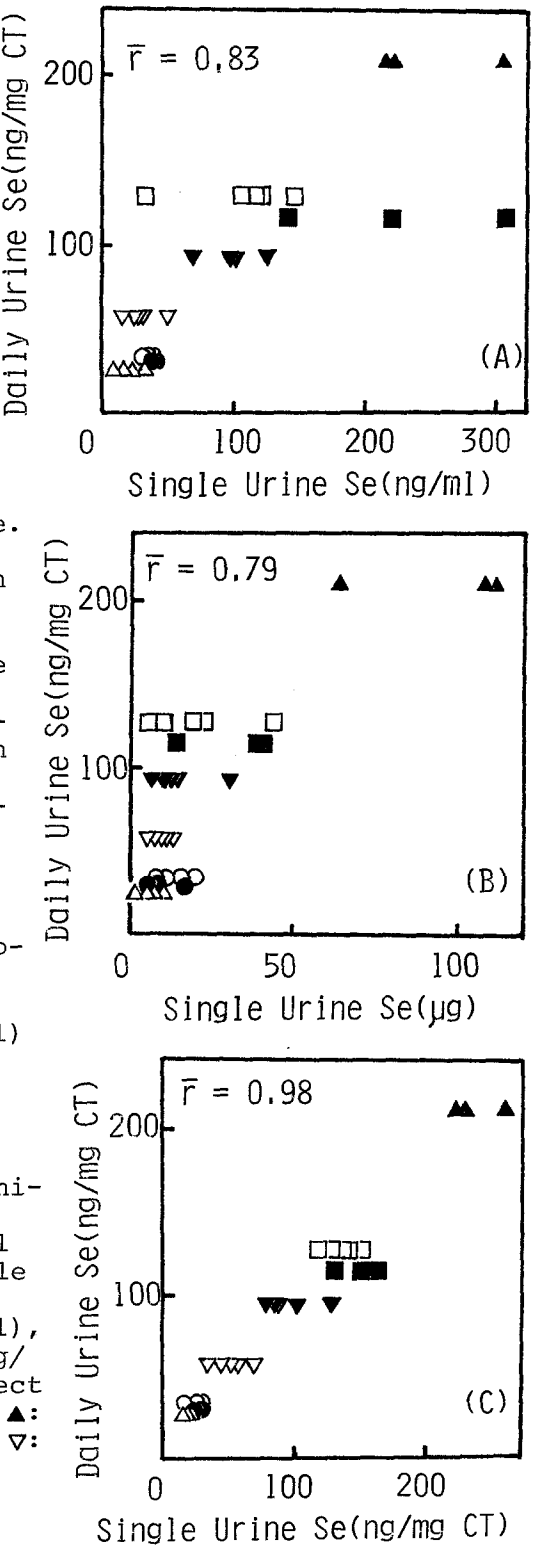


Fig.3. Se(ng/mg creatinine) level in 24-h urine plotted against Se level in individual void sample of a 24-h urine set. Se level is (A) Se(ng/ml), (B) Se(μg) and (C) Se(ng/mg creatinine). ○: Subject No.4. ●: No.5. △: No.6. ▲: No.7. □: No.8. ■: No.9. ▽: No.10. ▼: No.11. r̄: See Fig.1. CT: Creatinine.

The use of a single void urine sample as a substitute for a 24-h sample for urinary Se determination was examined. Fig.3 gives relationship between Se(ng/mg CT) in a 24-h urine and Se level in individual void sample of a 24-h urine set. The best correlation was obtained when Se(ng/mg CT) was used as Se level in a single void urine. The coefficients of correlation of single void urine Se(ng/mg CT), Se(μg) and Se(ng/ml) with 24-h urine Se(μg) were 0.88, 0.89 and 0.75, and those with 24-h urine Se(ng/ml) were 0.96, 0.80 and 0.92, respectively. These results show that single void urine Se(ng/mg CT) has the good correlation with all of indications of 24-h urinary Se level and particularly with Se(ng/mg CT). The single void urine, therefore, seems to be adequate as samples to estimate human Se status as substitutes for 24-h urines if Se(ng/mg CT) is employed as urinary Se levels.

Selenium levels expressed as Se(ng/ml), Se(μg) and Se(ng/mg CT) in a daily set of single void urines were compared. The mean, standard deviation and coefficient of variation of Se levels are shown in Table 3. A variable, Se(ng/mg CT) , was found to be much lower in

TABLE 3. Selenium levels[a] in individual void samples of a 24-h urine sample set.

Subject	Se (ng/ml)	Se (ng/mg CT[b])	Se (μg)	No. of samples
No. 4	32 ± 2 ($\overline{6}$)[c]	27 ± 6 ($2\overline{2}$)	14 ± 5 ($3\overline{6}$)	4
No. 5	37 ± 1 ($\overline{3}$)	29 ± 3 ($1\overline{0}$)	11 ± 5 ($4\overline{5}$)	3
No. 6	19 ± 11 ($5\overline{8}$)	26 ± 9 ($3\overline{5}$)	7 ± 3 ($4\overline{3}$)	4
No. 7	248 ± 50 ($2\overline{0}$)	240 ± 20 ($\overline{8}$)	95 ± 26 ($2\overline{7}$)	3
No. 8	105 ± 43 ($4\overline{1}$)	137 ± 12 ($\overline{9}$)	21 ± 15 ($7\overline{1}$)	5
No. 9	223 ± 83 ($3\overline{7}$)	151 ± 16 ($1\overline{1}$)	32 ± 15 ($4\overline{7}$)	3
No.10	30 ± 13 ($4\overline{3}$)	52 ± 13 ($2\overline{5}$)	10 ± 2 ($2\overline{0}$)	5
No.11	100 ± 20 ($2\overline{0}$)	96 ± 20 ($2\overline{1}$)	16 ± 9 ($5\overline{6}$)	5
Mean	99 ± 28 ($2\overline{9}$)	95 ± 12 ($1\overline{8}$)	26 ± 10 ($4\overline{3}$)	

a,b and [c]See Table 1.

TABLE 4. Correlation coefficient for Se levels in individual void samples of a 24-h urine sample set.

Subject	Se(ng/ml)-Volume(ml)	Se(ng/mg CT[a])-Volume(ml)	Se(μg)-Volume(ml)	Se(ng/ml)-CT(mg/ml)	N[b]
No. 4	0.61	0.50	0.99	-0.02	4
No. 5	0.69	0.69	1.00	-1.00	3
No. 6	-0.49	-0.63	0.57	0.87	4
No. 7	-1.00	0.34	1.00	0.96	3
No. 8	0.17	-0.38	0.80	0.98	5
No. 9	-1.00	-0.98	0.91	1.00	3
No.10	-0.98	0.59	0.67	0.95	5
No.11	-0.85	-0.29	0.99	0.41	5
Mean	-0.36	-0.02	0.87	0.52	
|Mean| [c]	0.72	0.55	0.87	0.77	

a,b and c See Table 2.

the degree of variation than Se(ng/ml) and Se(μg) as is evident from coefficient of variation. Table 4 and Fig.4 represent the effect of urine volume on Se levels. The data suggest that urine volume has slight influence on Se(ng/mg CT) and substantial influence on Se(ng/ml) and Se(μg). Good correlation between Se and CT concentrations shown in Table 4 and Fig.5 may underlie both the low degree of variation and the slight dependence on urine volume which were found in Se(ng/mg CT)of single void urines.These results suggest that Se(ng/mg CT) may possess advantage over Se(ng/ml) and Se(μg) as an indication of single urine Se levels in terms of variation and dilution effect.

DISCUSSION

Body samples hitherto used to assess human Se status are urine, blood(whole blood, erythrocyte and plasma), hair and nails. THOMSON & ROBINSON (1980) described whole blood and plasma Se have now replaced urinary excretion as an indicator of nutritional Se status in animals and man. But, VALENTINE et al.(1978) reported that it is not sufficient to use only blood as an indication of Se status, and that urine or hair measurements would be more likely to give valid results.

As indications of Se status using fluid samples, Se concentration has been largely used. To estimate urinary excretion of a given chemical substance without the influence of urine volume, recently, expression of urinary level of the substance as its concentration per creatinine concentration has been used (HUNTER et al. 1972, JAFFÉ et al. 1972, and VANDERLINDE 1979). Validity of this method, however, has never been exami-

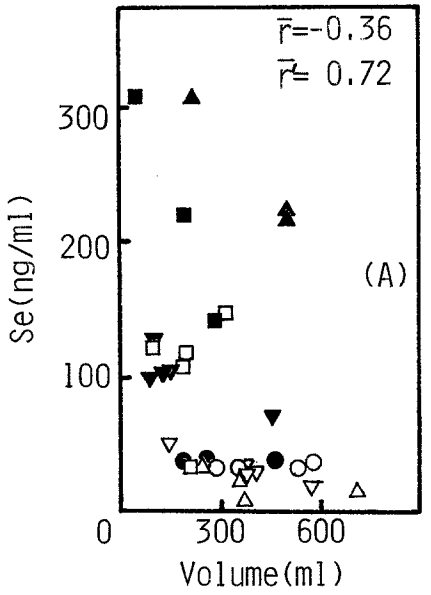
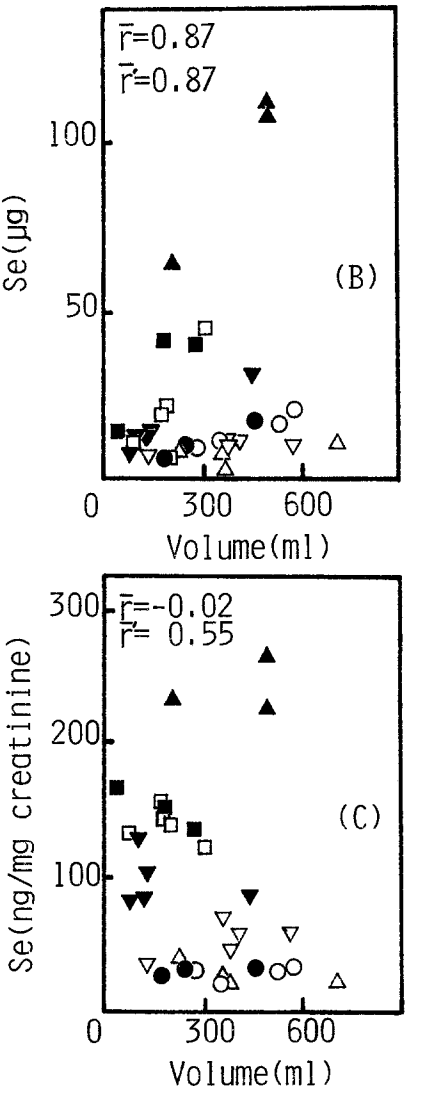


Fig 4. Selenium level plotted against volume in individual void sample of a 24-h urine sample set. Se level is (A) Se(ng/ml), (B) Se(μg) and (C) Se(ng/mg creatinine). $\bar{r}$ and $\bar{r}'$: See Fig.1. Other symbols: See Fig.3.



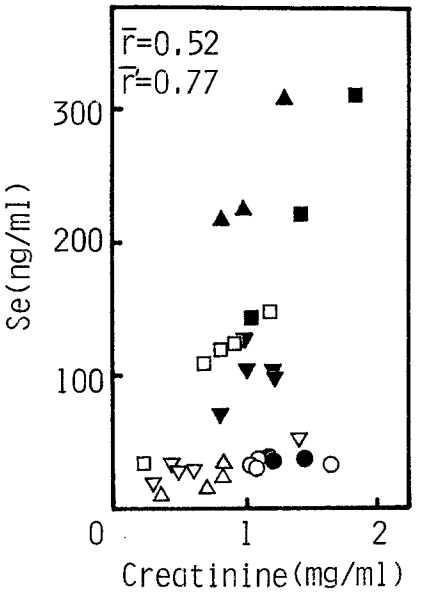


Fig.5. Selenium concentration plotted against creatinine concentration in individual void sample of a 24-h urine sample set. $\bar{r}$ and $\bar{r}'$: See Fig.1. Other symbols: See Fig.3.

ned particularly for Se excretion. It is the results in this study that Se(ng/mg CT) gives better constancy of Se excretion from single to single urines and from month to month in any one individual and is more nonsusceptible to dilution effect compared with Se(ng/ml). This clearly shows the advantage of expression of Se excretion as Se (ng/mg CT) over Se(ng/ml).

Use of single urine to estimate Se status has never been reported because single urine is subject to meal and dilution effects, but in this study was shown for the first time to be adequate. Single urine seems to have greater possibilities as samples to assess Se status in respect to the collection and preservation of large numbers of samples compared with daily urine.

Further examinations of expression of Se excretion as Se(ng/mg CT) are now in progress, involving the constancy from individual to individual and the relations with other monitors to assess Se status in comparison with Se(ng/ml).

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REFERENCES

BOSNES, R.W., and H.H. TAUSSKY : J. Biol. Chem. 158, 581 (1945).

BOWEN, H.J.M. : Trace Elements in Biochemistry. London-New York : Academic Press 1966.

BURK, R.F. : In, Trace Elements in Human Health and Disease. Vol.II. A.S. Prasad and D. Oberleas, Eds. New York : Academic Press 1976.

HUNTER, J., J.D. MAXWELL, D.A. STEWART, R. WILLIAMS, J. ROBINSON, and A. RICHARDSON : Nature 237, 399 (1972).

JAFFÉ, W.G., M. RUPHAEL-D., M.C. MONDRAGON, and M.A. CUEVAS : Arch. Latinoamer. Nutr. 22, 595 (1972).

THOMSON, C.D., and M.F. ROBINSON : Amer. J. Clin. Nutr. 33, 303 (1980).

VALENTINE, J.E., H.K. KANG, and G.H. SPIVEY : Environ. Res. 17, 347 (1978).

VANDERLINDE, R.E., F.J. KAYNE, G. KOMAR, M.J. SIMMONS, J.Y. TSOU, and R.L. LAVINE : In, Chromium in Nutrition and Metabolism. D. Shapcott and J. Hubert, Eds. Amsterdam : Elsevier/North-Holland Biomedical Press 1979.

VENUGOPAL, B., and T.D. LUCKEY : Metal Toxicity in Mammals 2. Chemical Toxicity of Metals and Metalloids. New York : Plenum Press 1978.

WATKINSON, J.H. : Anal. Chem. 38, 92 (1966).

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