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The assessment of biological variation components of copper, zinc, and selenium

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Twelve healthy volunteers (4 women and 8 men aged 23 to 45 years) were assessed for biological variation in copper, zinc, and selenium indices. Blood was drawn from these individuals once a week for 12 weeks and then once a month for a further 3 months. Over 12 weeks the mean plasma concentrations of copper, zinc, and selenium (measured by atomic absorption spectrometry), were 16 ± 2.9, 14.1 ± 1.5, and 1.28 ± 0.13 μmol/L, respectively. The intraindividual and interindividual coefficients of variation (estimated using analysis of variance (ANOVA) techniques) were 11 and 14% (zinc), 8 and 19% (copper), and 12 and 14% (selenium), respectively. The analytical goal for imprecision was achieved for all three micronutrients, i.e., it was less than one-half of the measured intraindividual variation. The indices of individuality for all three elements indicated that an individual's results as reference values are more useful than population-based data. The critical difference for significance between serial results is relatively smaller for copper (23%) and zinc (30%) than that for selenium (35%). The monthly mean concentrations of the three micronutrients over 6 months demonstrated no seasonal pattern. (J. Nutr. Biochem. 6:43–47, 1995.)

Keywords: intraindividual variation; interindividual variation; copper; zinc; selenium

Introduction

Copper, zinc, and selenium are essential trace metals involved in multiple biological processes[1–3] as constituents of enzyme systems including superoxide dismutase, oxidoreductases, and glutathione peroxidase. Blood levels of copper, zinc, and selenium have been monitored in several experimental nutrition studies and are widely used to determine the presence of deficiency states or toxicity. Interpretation of measured values depends on accurate estimates of the usual concentrations of these substances in blood and their normal variance. Estimation of components of biological and analytical variation are extremely useful in determining goals for analytical performance, evaluation of the significance of a change between two measurements in an individual, and the usefulness of a population reference range. The within-subject and between-subject biological variations of these quantities have not been investigated in depth as shown in collated data,[4] in spite of the growing interest in the value of measuring their levels. We therefore investigated analytical and biological components of variation of these mineral nutrients.

In this study we monitored the distribution of copper, zinc, and selenium levels in a group of 12 healthy individuals over a period of 6 months. The magnitude of intraindividual variation of these elements and the relationship between intraindividual and interindividual components of variation were determined.

Methods and materials

Subjects

Twelve healthy volunteers, 4 women aged 24 to 43 years, and 8 men aged 23 to 45 years, were studied. The participants maintained their lifestyles throughout the study period; none were taking nutritional supplements or any drugs, including oral contraceptives. No restrictions were imposed on diet or activity during the study. The last meal before blood sampling was between 1800 and 2000 hr the night before. No food (except water) was consumed after midnight until blood collection the next morning, before breakfast. All subjects maintained their usual dietary pattern for the 24 hr preceding blood sampling for the duration of the

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study. All gave informed consent to the protocol which had been approved by our institution's Ethics Committee.

Venous blood was collected over 6 months, every week for the first 12 weeks, and then monthly for 3 months. The individuals rested for 10 min in a sitting position and a tourniquet, applied briefly, was released before venipuncture. Blood was drawn by an experienced phlebotomist between 0800 and 1000 hr, on the same day of the week, into heparinized vacutainers. An aliquot of whole blood was kept at 4° C for selenium analysis before specimens were centrifuged at 1500*g* for 10 min within half an hour of collection, and the plasma was separated and stored at 4° C until analysis. This protocol minimized preanalytical variation.

Measurements of trace metals

Copper and zinc were determined in plasma by an air-acetylene flame atomic absorption procedure (Varian Spectra-20, Varian, Australia). Calibration is based on standard solutions at levels of 17 μmol/L for copper and of 9.5 and 19.0 μmol/L for zinc. Selenium was determined in plasma and whole blood by a hydride generation atomic absorption procedure. Selenium levels were calculated by proportionality using standard solutions equivalent to 0.64 and 2.54 μmol/L. Samples and quality control specimens were analyzed in random order during each run. The analytical coefficient of variation was calculated from the values obtained by measuring the same control solution with each batch of specimens analyzed.

Statistical analysis

Using analysis of variance techniques (ANOVA) (Minitab statistical software) we calculated the following components of variation: (1) the average variation of true means (or homeostatic set points) among subjects was estimated as the coefficient of variation of the individual means of all volunteers (CV_G or interindividual coefficient of variation); (2) for each volunteer the within-subject biological variance was estimated, that is the variance of true values around the homeostatic set point from time to time in the subject (CV_I or intraindividual coefficient of variation); and (3) the CV_A or between run analytical variation over the whole of the study period. These components of variation were used as suggested by Fraser and Harris[5] to calculate the analytical goals for imprecision (0.5 CV_I) and bias 0.25 $(CV_I^2 + CV_G^2)^{1/2}$. To assess the utility of conventional population-based reference values an index of individuality was calculated as CV_I/CV_G. The critical difference, i.e., the difference in concentration required for two serial results to be significantly different (at $P \leq 0.05$), was calculated as $2.77(CV_A^2 + CV_I^2)^{1/2}$. The heterogeneity of the within-subject variation (index of heterogeneity) was calculated as the observed CV of a set of individual variances to the theoretical CV, namely $(2/[n-1])^{1/2}$ where n is the average number of observations per subject; significant heterogeneity exists when the index differs from 1.00 by more than 2SD, defined by $2/(2n)^{1/2}$.

Results

A total of 144 specimens were obtained for trace metal levels in 12 subjects during the first 12 weeks. Using Minitab we inspected the distribution of the data collected for conditions that would have affected the validity of the statistical analysis. The estimation of variance components through ANOVA is a valid procedure regardless of the mathematical form of the distribution of observations.[5] However, for purposes of homogeneity, highly deviating values (outliers) should be deleted. No outliers were found so all results were used for estimating the components of weekly biological variation over this period. A total of 84 specimens were obtained for calculation of the monthly variation over the 6 month study period from these 12 subjects. No outliers were found and, thus, all values were used to calculate the components of monthly variance. Descriptive statistics such as means and standard deviations were calculated on the original data.

A summary of the average of the individual means of all volunteers, the analytical variance (V_A), the intraindividual variance (V_I), the interindividual variance (V_G), and the corresponding coefficients of variation for the 12 week study period is shown in *Table 1*. Analytical goals for imprecision and bias, indices of individuality, critical difference ($P \leq 0.05$), and indices of heterogeneity are shown in *Table 2*.

Figure 1 demonstrates the intraindividual variation of plasma copper and zinc in the first 12 weeks. The levels of these elements in each subject in the 6 month period was in a similar range with that in the first 12 weeks (data not shown).

Figure 2 demonstrates the intraindividual variation of plasma and whole blood selenium in the first 12 weeks. Selenium levels over the 6 month study period were in a similar range (data not shown).

The mean plasma concentration of copper in all 12 subjects by month of blood sampling was in the range of 13 to 25 μmol/L, for plasma zinc it was 13 to 17.5 μmol/L, for plasma selenium it was 1.2 to 1.6 μmol/L, and for whole blood selenium it was 1.4 to 1.9 μmol/L. No clear seasonal pattern was demonstrated for any of these elements.

Discussion

Evidence suggesting that micronutrients may modify the risk of certain diseases, such as cardiovascular disease, inflammatory disease, and some types of cancer,[6] has stimulated studies involving measurement of these micronutri-

Table 1 Mean values and components of variation for zinc, copper, and selenium over 12 weeks

Quantity	Mean*	V_A*	CV_A%	V_G*	CV_G%	V_I*	CV_I%
Zinc	14.1 ± 1.5	0.31	2	4.85	14	3.24	11
Copper	16.0 ± 2.9	0.35	2	9.85	19	2.49	8
Se	1.28 ± 0.13	0.003	4	0.035	14	0.026	12
Se-Wb	1.58 ± 0.13	0.003	3	0.043	12	0.043	12

*Units are in μmol/L.

Table 2 Analytical indices and critical difference (CD) for plasma zinc, copper, selenium, and whole blood selenium (Se-Wb)

Quantity	Analytical goal imprecision %	Analytical goal bias %	Index of individuality	CD%	Index of heterogeneity
Zinc	5.5	4.4	0.78	30	1.7
Copper	4	5.1	0.42	23	1.7
Se	6	4.6	0.85	35	1.01
Se-Wb	6	4.2	1.0	35	1.01

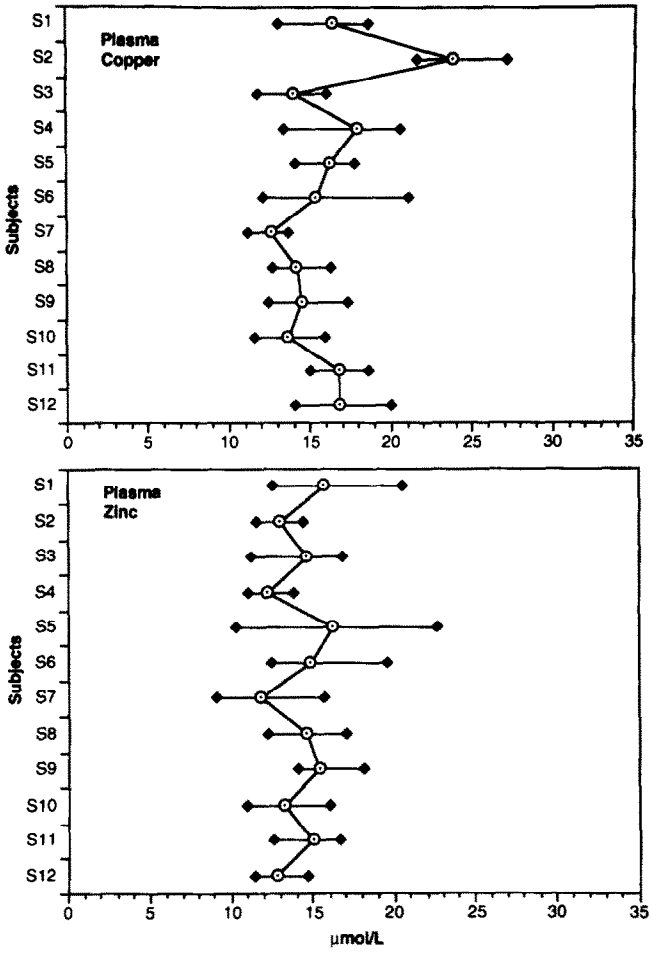

Figure 1 Means and ranges for plasma copper and zinc concentrations (µmol/L) in 12 healthy individuals over 12 weeks.

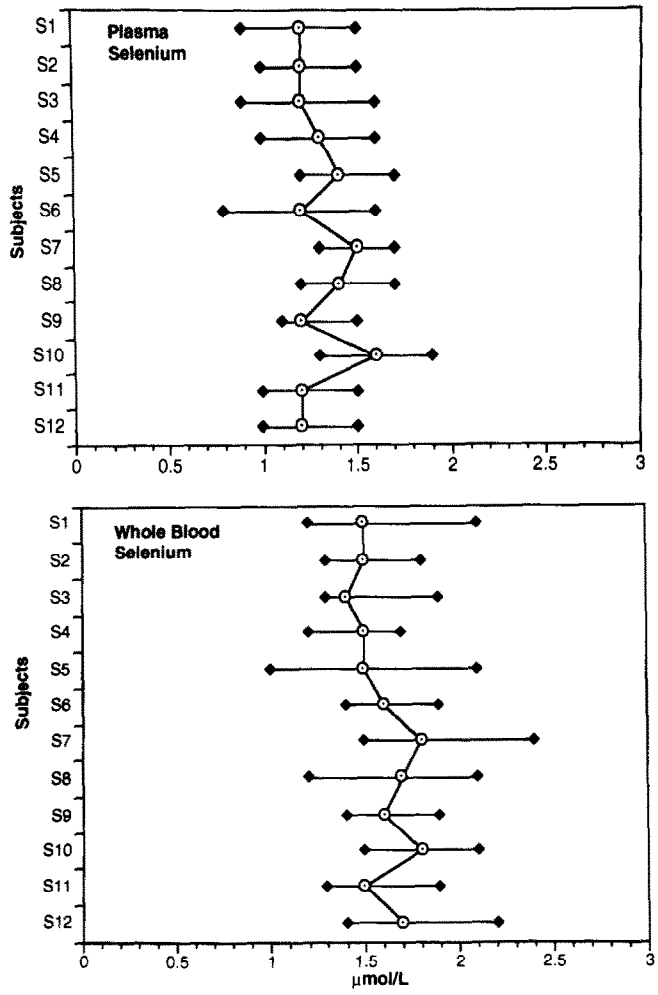

Figure 2 Means and ranges for plasma and whole blood selenium concentrations (µmol/L) in 12 healthy individuals over 12 weeks.

ents in blood specimens. Assuming that the physiological functions of micronutrients are related to their concentrations in body fluids, knowledge of the variation of those concentrations over time is essential before contemplating therapeutic intervention and also for the interpretation of epidemiological studies of these trace metals. A review of the literature indicated that there is limited information regarding intra- and interindividual variation for copper, zinc, and selenium.[4,7] This study was designed to estimate the components of variation and to set analytical goals for the measurement of these quantities.

Analytical attributes of a test are critically important in determining whether the test will be useful in distinguishing a normal from an abnormal result, or to determine whether a day-to-day change in test results is significant. The analytical goal for imprecision, based on biological variation, should be equal to or less than one-half of the measured day-to-day intraindividual variance. The analytical goals for imprecision for all three micronutrients were easily achieved in this study. Previous studies[4,8] have reported a similar analytical imprecision for plasma zinc and copper of 2%, as in this study. However, intraindividual coefficients of variation (CV_I) from as low as 2%[4] to approximately 10%[8] were reported. The intraindividual variation observed

in this study (*Table 1*) is likely to be a more realistic estimate of the true biological variation because it is consistent with current achievable analytical imprecision.

With regard to the assessment of intraindividual variation, two important issues were considered in designing this study: the effect of meals and the time of collection of blood samples. Copper and selenium levels show no diurnal variation and are unaffected by meals while zinc levels show diurnal variation, being highest in the morning and lowest at the end of the day.[7] Furthermore, Hambidge et al.[9] have shown that plasma zinc concentrations are affected by the time and size of meals consumed prior to taking blood samples. Giving standard meals to subjects on the evening prior to blood sampling would be essential in a study of zinc metabolism, especially where the effect of meals was being investigated. However, this is not practical in routine diagnostic laboratory medicine or in epidemiological studies and thus our protocol sought to cause minimum disruption to the individual's lifestyle. The usual evening meal was eaten between 1800 and 2000 hr, with a minimum fasting period of 8 hr prior to taking blood samples in the morning, at the same time (and day of the week) on each occasion, and before breakfast. This is a pragmatic approach which closely reflects current routine practice in our diagnostic laboratory and, probably, in most similar laboratories in the world. Our finding of a mean intraindividual variation of 11% for zinc is thus of particular value for the interpretation of serial zinc levels obtained prebreakfast, after an overnight fast. It is difficult to compare the intraindividual variation of 7.8% reported by Hambidge et al.[9] with that observed in our study. They used standard meals and obtained four samples over 3 to 5 weeks, with a different dietary protocol before each sample was obtained, while in our study samples were obtained weekly over 12 weeks and monthly over 6 months, following a nonstandard meal the night before. Nevertheless, the study by Hambidge et al. would suggest that some component of the intraindividual variation observed in our study may be due to variation in the size and content of the meal consumed the night before.

Serum and whole blood selenium measurements were reported to have analytical imprecision of 3.8% and 4.5%, respectively,[10] which is similar to the imprecision during our study. The average intraindividual coefficient of variation of selenium over 12 weeks was 12%. To our knowledge there are no previous studies on long-term biological variation of selenium. A recent report on diurnal variation in Japanese male adults[7] found an intraindividual coefficient of variation of 5.2% over 24 hr. The interindividual coefficient of variation (15.1%) was similar to that in our study (14%).

The analytical goal for bias calculated for copper and zinc (*Table 2*) was achieved by our current analytical methods. Participation in the Trace Elements Quality Assessment Scheme (TEQAS, Surrey, U.K.) demonstrated that our method for copper determination has an average bias of 4.9%. Eighteen out of the 119 participating laboratories showed equal or better performance. The method for zinc determination has a bias of 4.4% (TEQAS), which is equivalent to the analytical goal (*Table 2*). Fourteen out of 131 participating laboratories showed equal or better performance. Our method for selenium had a bias of 8.3% which is greater than the analytical goal of 4.6% (*Table 2*). Thirty-seven out of 48 participating laboratories showed an equal or worse performance. Thus the accuracy of selenium measurement by most laboratories in the world, including our own, requires improvement in order to achieve the desired analytical goal.

In this study we have used data from 12 subjects. Several specimens need to be collected from an individual in order to determine the homeostatic set point for a particular analyte. The degree of error in determining that point for each individual can be calculated by rearranging the usual standard error of the mean formula,[5] $n = (Z^*CV_{A+I}/D)^2$, where n is the number of specimens, Z the number of standard deviates required for a stated probability under the curve, and D is the percentage closeness to the homeostatic set point. Using a value of 1.96 for Z (95% confidence) we find that with 12 specimens our estimate of the true homeostatic set point of each subject would have a 5% error for copper, 7% for zinc, and 9% error for selenium. Reducing the number of specimens obviously increases the error, and thus, a single measurement of a subject's plasma level of these trace elements is an imprecise estimator of the true homeostatic set point.

Conventional population-based reference values are of utility only when the within-subject variability exceeds the between-subject variability. The ratio of the intraindividual coefficient of variation to the interindividual coefficient of variation is called the index of individuality. Only when this index (CV_I/CV_G) is larger than 1.4 are population-based reference values useful.[5] Indices of individuality over our study period were less than 1.4, indicating an individual's results as reference values are more useful than population-based data. The high degree of individuality indicates that subjects would have levels that are very unusual for them, but these would still lie within the population-based reference limits. It is therefore preferable that the subject's previous values be used as a guide to interpreting any future value.

Difference in serial results are due not only to the disease process but also to preanalytical, analytical, and within-subject sources of variation. Intraindividual variation contributes to the critical difference (CD) for significance between serial results. Our studies show that a relatively small difference between results from sequential specimens is required for copper (23%) and zinc (30%) to be significantly different than that for selenium (35%). Use of the average CV_I to calculate critical differences is truly valid only if all subjects have a similar CV_I. This was investigated using an index of heterogeneity of within-subject variation. When the index is more than 1.45, then significant heterogeneity exists and the calculated critical difference is valid. As shown in *Table 2*, this is the case for copper and zinc, but not for selenium. However, we believe that the critical difference detailed here for plasma and whole blood selenium will provide some clinical guideline in the interpretation of changes in serial results.

An interesting finding was that whole blood selenium, which is usually regarded as a better index of body selenium status than plasma selenium because it is a measure of intracellular selenium, in fact showed greater intraindividual variance (V_I). It is therefore questionable whether whole

blood selenium has any advantage over plasma selenium in assessing an individual's selenium status.

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