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Intraindividual Variability in Serum Micronutrients:

Effects on Reliability of Estimated Parameters

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Abstract

Background: Although serum measures of micronutrients are more specific than questionnaires in quantifying nutritional status, the reliability of serum measures depends on between- and within-person variability of circulating micronutrient levels. The extent to which multiple samples per person might improve reliability is useful information for planning studies and interpreting results.

Methods: We analyzed levels of 25 micronutrients in serum samples taken from 381 Hawaii women at 4-month intervals. For all subjects and for subjects at the low and high end of the micronutrient distributions, we calculated inter- and intraindividual variability, reliability coefficients, and the number of measurements required to limit attenuation in estimated parameters (ie, to keep estimates close to their true values).

Results: For 18 of the 25 micronutrients, a single measurement provided an estimate within 20% of the true value. For regression coefficients, 2 measurements were needed to limit attenuation to no more than 20% for nearly half of the micronutrients. To achieve no more than 10% attenuation, the number of measurements required ranged from 2 to 10 for correlation and from 3 to 20 for regression coefficients. To achieve no more than 5% attenuation, the corresponding ranges were 3 to 21 for correlation and 6 to 42 regression coefficients. In general, more measurements were required for adequate characterization of subjects with relatively high levels of micronutrients than for subjects with lower levels.

Conclusions: Our analysis suggests that 2 or 3 blood measurements are enough to limit attenuation of regression coefficients within 20% of the true value for most carotenoids and tocopherols. For 10% attenuation or less, 4 or more micronutrient measurements may be needed.

Investigators have reported on the role of micronutrients (specifically circulating and dietary carotenoids and tocopherols) in the etiology of various types of cancer.[1] Results of such studies have not been entirely consistent, with some studies reporting inverse associations of micronutrients such as β-carotene with cancer risk, whereas others suggest positive associations.[2,3] These inconsistencies could be due to various factors, one of which is imprecision from intraindividual variability in micronutrient levels.

Circulating micronutrient levels differ among individuals by various factors including age, sex, ethnicity, genetics, physiology, lifestyle (eg, alcohol use and tobacco smoking) and dietary habits.[4,5] Within-person variability of circulating micronutrient concentrations can be caused by environmental exposures, changes in daily diet, weight, physical activity, and seasonality.[6-8] The extent of such within-person variability also depends on the micronutrient under consideration. For example, micronutrients derived from common food sources or that have longer half-lives in circulation would be expected to exhibit less within-person variation. On

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the other hand, some foods are consistently consumed by certain individuals but not others, thereby affecting within-person variability even in otherwise homogeneous populations.

Inter- and intraindividual variability can greatly affect the results of a statistical analysis. A common assumption is that the observed blood levels of micronutrients vary randomly about a long-term average level. Measurement of micronutrient concentrations in a single blood sample per individual to characterize the average level among participants in epidemiologic studies might result in substantial attenuation in the estimated parameters.[7] One way to improve the accuracy of the statistical estimates is to increase the sample size, thereby reducing the effect of overall variability. Another way is to decrease the influence of intraindividual variability by increasing the number of measurements taken from each participant.

The influence of intraindividual variability in the data can be quantified by the attenuation coefficient, which measures the degree to which the estimated parameter differs from its true value, that is the value obtained in the absence of intraindividual variation. Liu et al[9] and Keys[10] have discussed a formula for the estimated correlation coefficient compared with the true correlation ρ_{XY}: $\rho_{XY}/\left(1+\sigma_w^2/\sigma_b^2\right)^{1/2}$, where σ_b^2 denotes the variance between group means and σ_w^2 the variance within groups. An important question for researchers is how to determine the number of measurements necessary to limit attenuation to some acceptable level. The answer depends on the relative size of inter- and intraperson variability. In the aforementioned formula, the expression $1/\left(1+\sigma_w^2/\sigma_b^2\right)$ is referred to as the intraclass correlation (or reliability) coefficient. It measures the fraction of total variance that is attributable to variance between group (subject) means.

The objective of this analysis is to compute the estimates of inter- and intraindividual variance components and intraclass correlation coefficients for 25 plasma micronutrients, using well-controlled, standardized procedures during the collection, processing, storage, and analysis as a means of restricting external variability. We also present the number of observations necessary to limit attenuation in each of the micronutrients to some acceptable level, as well as an estimate of how reliable the data would be if just 1 observation of a micronutrient level were taken.

METHODS

Sample Population and Study Design

In this analysis, we used 1502 blood samples obtained from 381 women participating in a cohort study of cervical human papillomavirus conducted at the Cancer Research Center of Hawaii.[11] To be eligible, women had to be 18 years or older and residents of Oahu. Women who had undergone hysterectomy, who were pregnant within the past year or diagnosed with cervical squamous intraepithelial lesion within the past 18 months were ineligible. Subjects were recruited at 5 clinics in Honolulu between July 1999 and December 2003 and followed with repeated visits at 4-month intervals. All subjects completed 2 to 16 visits. At baseline, a questionnaire was administered by an interviewer to collect information on social and demographic factors, medical and sexual history, and tobacco and alcohol use. At each visit, a blood sample was obtained and a questionnaire updating information on tobacco and alcohol use was conducted. The study was approved by the Committee on Human Subjects of the University of Hawaii.

Laboratory Procedures

Blood was drawn at the clinic after a 10- to 12-hour fast and was used to determine plasma levels of both *cis* and *trans* forms of lutein/zeaxanthin, lycopene, total cryptoxanthin, α-

cryptoxanthin, β-cryptoxanthin, total carotene, α-carotene, β-carotene, retinol, total tocopherol, α-tocopherol, β- and α-tocopherol, and δ-tocopherol. Specimens were wrapped in foil for light protection immediately upon blood draw, and all processing was conducted in the dark. The plasma was separated from the cells by centrifugation (4°C for 15 minutes, 1800*g*) and frozen (−80°C) until analyzed. The median time from blood draw to freezer was less than 2 hours. Sample extracts were analyzed isocratically by reverse-phase high performance liquid chromatography (HPLC) with photodiode array detection.[8,12] Absorption spectra and retention times for each peak were compared with those of known authentic standards. Briefly, under subdued light, 0.5 mL of thawed plasma was vortexed by hand for at least 1 minute with 0.5 mL of ethanol containing 0.3 mg/L *n*-butyl-, β-apo-8′-carotenoate (as an internal standard for carotenoids), 1.0 mg/L retinyl laurate (as an internal standard for retinoids), 6.0 mg/L tocol (as an internal standard for tocopherols), and 250 mg/L butylated hydroxytoluene (as preservative) followed by extraction with hexane. After drying the hexane extracts with nitrogen at ambient temperature and redissolving it in mobile phase, 0.01 mL was subjected to HPLC analysis. The quality of all laboratory analyses was evaluated by participation in the quality assurance program for lipid soluble vitamins organized by the US National Institute for Standards and Technologies (Gaithersburg, MD) and by comparison with external standards (pooled plasma from 8 healthy volunteers). The validation results were within 1 standard deviation from the true value. Interassay coefficients of variation were not more than 17% for *trans*-zeaxanthin, 37% for δ-tocopherol, 12% for retinyl palmitate, and within 5% for the other 22 micro-nutrients. Intraassay coefficients of variation were between half and two thirds of those for interassay variation.

Statistical Analysis

Statistical analyses were performed using SAS version 9.1.3 (SAS Institute Inc., Cary, NC). Means, medians, ranges, and standard deviations were computed for untransformed micronutrient levels. As noted by Morgan et al,[13] individuals with higher mean nutrient levels also tend to have higher within-person variability. For this reason, we scaled and log-transformed the data before proceeding with the remaining computations. Scaling the data allowed comparisons between micronutrients, and log transformation normalized the data. Between- and within-person variances were determined through mixed-model analysis using by restricted maximum likelihood methods and adjusting for age, ethnicity, smoking, and drinking habits. The intraclass correlation coefficient (ICC) was calculated as a ratio of between-person and total variances. Similar to Block et al,[14] we calculated the number of measurements required to limit attenuation to 5%, 10%, and 20% of the true value in correlation coefficients, where correlation of a micronutrient level and another factor, measured without error, is investigated, and in regression coefficients, where a nutrient level is an independent factor. Liu et al[9] and Sempos et al[15] provided the following formulas for the required number of measurements:

$$n_\rho = \frac{P^2}{1-P^2}\frac{s_w^2}{s_b^2}, \quad n_\beta = \frac{P}{1-P}\frac{s_w^2}{s_b^2}; \tag{1}$$

where n_ρ, n_β denote the number of measurements to limit attenuation in correlation and regression coefficients, respectively; s_w^2, s_b^2 denote the observed within- and between-person variances, respectively; and P equals 1 minus the maximum allowed attenuation, expressed as a fraction of 1. Fixing $n = 1$ and solving for P, we obtain formulas for attenuation (denoted λ_ρ for correlation and λ_β for regression) that results from using a single measurement:

$$\lambda_\rho = \frac{1}{\sqrt{1+\left(s_w^2/s_b^2\right)}}, \quad \lambda_\beta = \frac{1}{1+\left(s_w^2/s_b^2\right)}. \tag{2}$$

These 2 attenuation coefficients can serve as a measure of reliability of the nutrient data. They value between 0 and 1, implying substantial or little attenuation, respectively.

To assess the effect of seasonality on within-person variation, a season effect was introduced. To account for different seasonal patterns in levels of various micronutrients, we defined a season as 3 consecutive months, rotated through all 12 months, compared with the rest of the year. The ICCs and attenuation factors were also computed for the extreme quartiles of distributions of the 25 micronutrients, using a model with 3 variance components (between-quartile, between-person within quartiles, and within-person). To prevent artificially deflating the between-person variance due to quartile stratification, this variance component was estimated as a sum of between-quartile variance and the between-person variance within quartiles. Quartile classification was based on the log-transformed micronutrient levels.

RESULTS

An average of 4 blood specimens was collected from each of the participants during an average of 1.25 years of follow-up (Table 1). The mean age of the participants at first visit was 30 years. The majority of women were nonwhite. Alcohol consumption and tobacco smoking were uncommon.

The intraclass correlation coefficients for the 25 plasma micronutrients were generally greater than 0.6 (Table 2). Age adjustment made little difference to the ICCs (data not shown). About 3 to 21 micronutrient measurements were required to limit attenuation in ICCs to 5%, 2 to 10 measurements were required for <10% attenuation, and 1 to 4 measurements were required for <20% attenuation. In the latter case, the only micronutrients requiring more than 1 measurement were lycopene (including total and *dihydro*-lycopene) δ-tocopherol, and retinyl palmitate. These were the only micronutrients with ICCs of 0.5 or lower, and they consistently required more measurements than other micronutrients. Attenuation factors for correlation coefficients resulting from the use of a single measurement ranged from 0.56 to 0.88. Lycopene (including total and *dihydro*-lycopene) δ-tocopherol and retinol palmitate were the only micronutrients with attenuation factor below 0.7.

The number of measurements required to limit attenuation in regression coefficients was 6 to 42 for <5% attenuation, 3 to 20 for <10% attenuation, and 1 to 9 for <20% attenuation (Table 2). Again, the aforementioned 5 micronutrients required the greatest number of measurements (≥5). At 20% attenuation, nearly half of the micronutrients required 1 or 2 measurements. The attenuation factors for regression coefficients resulting from a single measurement ranged from 0.31 to 0.78.

Seasonal variation accounted for a small fraction of within-person variation: less than 7% for α-carotene and less than 3% for all other micronutrients (data not shown). Analyses were also repeated after omitting the 48 (13%) women who changed their smoking habits during follow-up. The results were nearly identical for all micronutrients except *dihydro*-lycopene, for which the ICC increased from 0.43 to 0.57 and the required number of measurements for regression coefficients dropped nearly 2-fold (from 12 to 7 for <10% attenuation and from 6 to 3 for <20% attenuation; data not shown).

The ICCs based on the high quartile of micronutrient distribution were lower than those based on the low quartile (Table 3). For subjects at the low end of the micronutrient range, 1 measurement was required for <10% attenuation in both correlation and regression coefficients for nearly half of the micronutrients. All micronutrients required a single measurement at 20% attenuation. For subjects whose levels were above the 75th percentile of circulating micronutrient concentrations, the number of measurements required for <10% attenuation was 1 to 11 for correlation and 2 to 23 for regression coefficients; and 1 to 5 and 1 to 11, respectively, for <20% attenuation.

The analysis in Table 2 was repeated separately for younger (<45 years) and older (≥45 years) women. For all micronutrients except retinyl palmitate, the ICCs for the older group were slightly higher than the ICCs for the younger group. For correlation coefficients, the required number of measurements to limit attenuation to 20% was reduced for 6 micronutrients, whereas for regression coefficients, the corresponding number of measurements was reduced for 15 micronutrients as compared with the overall population (data not shown).

DISCUSSION

Our findings suggest that a single measurement of circulating carotenoids and tocopherols is often not enough to characterize adequately an individual's average blood levels within 20% of their true level. At least 1 or 2 measurements are required to limit attenuation in correlation coefficients to 20%; and 2 to 3 measurements are required for <10% attenuation. Because the 10% attenuation limit would likely be acceptable in most epidemiologic studies, a study design with 2 to 3 micronutrient measurements per subject would generally resolve the attenuation problems resulting from within-person variability. For regression coefficients, the number of measurements required to adequately characterize an individual is nearly twice that for correlation coefficients. About 2 to 4 measurements would be sufficient for all but 5 micronutrients to bring attenuation within 20% of the true value. With a requirement of 10% attenuation, this is true for only half of the micronutrients. In a study in which only 1 measurement is used, great care should be exercised in interpreting results of no association. Such results could be caused by attenuation in true parameter values, even when a substantial association does exist (type II error). We note, however, that the improvement in parameter precision with repeated measurements comes at the expense of increased cost and more complex logistical issues associated with multiple specimen collection.

Our results also suggest that for a group of individuals at the high end of micronutrient range, the proportion of the total variability that is due to within-person variability is greater. Thus, this group may require more observations. This becomes increasingly important if the desired maximum attenuation is 10% or less. This observation may be useful to an investigator designing an intervention study that includes initial screening, in which only the subjects with high (or low) initial screening levels are entered into the study.

The difference in the number of measurements required to limit attenuation in regression and correlation coefficients follows directly from the difference in attenuation factors λ_ρ and λ_β Eq. (2): λ_β depends on the within-person variability of the micronutrient alone, whereas λ_ρ depends on within-person variability of both the micronutrient and another factor. Assuming, as we have done in this study, that the other factor is error free, the correlation coefficient is attenuated less than the regression coefficient and thus requires fewer measurements of the micronutrient to keep it within a certain percentage of its true value.

The notable exceptions to our findings are for lycopene (including total and *dihydro*-lycopene) δ-tocopherol, and retinyl palmitate. These 5 micronutrients had the lowest intraclass correlation coefficients among the 25 micronutrients, which imply that their intraindividual variance is

highest as a fraction of total variance. Consequently, they require a higher number of measurements to limit the influence of within-person variation and to lower attenuation. This finding results from the shorter half-life of these micronutrients in circulation. Schwedhelm et al[16] reported half-life of 2 hours for δ-tocopherol, 13 hours for α-tocopherol, 73 to 81 hours for α-tocopherol, 2 to 3 days for lycopene and 5 to 7 days for β-carotene. Burri et al[17] determined that in persons on carotenoid depletion diets, the half-life of lycopene is 26 days, whereas the half-lives of lutein, α- and β-carotene, cryptoxanthin, and zeaxanthin are between 37 and 76 days. The half-life of retinyl palmitate has been estimated to be less than 2 hours.[18] Our results indicate that δ-tocopherol has the smallest ICC among tocopherols, and lycopene the smallest ICC among carotenoids. Although α- and β-tocopherol have shorter half-lives than most carotenoids, their ICCs are comparable with those of other carotenoids with the exception of lycopene. This could be because tocopherols come from more stable food sources, which results in continued supply of these substances and reduces within-person variation in blood levels.

A number of past studies could have benefited from taking 2 or more micronutrient measurements per person. This is especially true of prospective cohort studies, in which participants are followed over a long period of time. It is not uncommon for such studies to use 1 plasma micronutrient measurement per person. In some cases, no association has been found between cancer risk and such micronutrients as lycopene, retinol, and tocopherols. These null results could have been caused by attenuation due to the use of a single measurement. Methods exist[19,20] to construct unattenuated parameter estimates with only 1 measurement per person, but such methods require a good estimate of within-person variance in the target population of the study. In the absence of such estimates, the attenuation factors reported here could be used to perform a sensitivity analysis and to determine the potential influence of attenuation on the study results.

Few investigators have examined the intra- and interindividual variability of circulating micronutrient concentrations. Tangney et al[21] compared within- and between-person variances of fasting venous blood samples taken from 24 male medical staff once each week for 4 consecutive weeks. The number of measurements to attain 10%, 20%, and 40% attenuation was calculated for β-carotene, retinol, α-tocopherol, and total tocopherol. The numbers reported were 3, 4, 8, and 8, respectively, at 10% attenuation, which is generally higher than our results (2-4 at 10% attenuation). Similar to our results, β-carotene required only 1 measurement at 20% attenuation.

Cooney et al[8] measured plasma levels of 12 micronutrients in samples from 21 persons taken at monthly intervals over a 1-year period, and calculated the number of measurements required to characterize accurately long-term plasma levels at least 80% of the time. The numbers reported for lutein/zeaxanthin, α-carotene, and retinol (3, 4, and 8.3, respectively) were substantially higher than our results (1, 1, and 2, respectively), whereas those for lycopene, β-carotene, α- and β-tocopherol (3, 1, 1.5, and 2.6, respectively) were similar to our findings.

In a much larger study of 206 primary and second-hand tobacco smokers, Block et al[14] measured micronutrients in 2 fasting blood samples from each participant separated by 2–4 week intervals. Reliability coefficients and attenuation factors calculated for 25 micronutrients (generally overlapping with ours) were obtained through a different estimation method and were comparable with those in our analysis, but generally higher than those reported by Tangney et al[21] Block et al[14] concluded that 2 measurements were adequate for a reliable dataset.

There is ongoing debate as to whether standard methods of handling measurement error can be applied to the intraindividual variability in micronutrient levels because such variability

may not be random. Morgan et al[13] described substantial autoregressive trends in daily nutrient intakes that might be explained by temporal factors. In an analysis of variability in daily food records, Sempos et al[15] argued against strong influence of long-term changes in diet on within-person variance. Rather, short-term dietary variation, including seasonal influence on food choices, appears to have a stronger influence on micronutrient intake. In the present study, however, seasonality was not an important source of variability in serum micronutrient levels, generally accounting for less than 3% of intraindividual variation. This might be because of the general availability of major food sources throughout the year in Hawaii, which is not true in all populations. In our cohort and others in which seasonality is not important, sample collection from the same person could be spaced several days or weeks apart for more precise micronutrient classification. In populations with strong seasonal variations in diet, samples should be taken several months apart to capture seasonal effects.

The ICCs for serum micronutrients depend on inherent characteristics of micronutrients (such as half-life in circulation) as well as dietary factors, age, sex, ethnicity, geographic location, and seasonality. Our results may not be generalizable to other populations with different distributions of micronutrient levels, as the ICC is affected by the interindividual variability within a population. Thus, the number of required measurements may vary depending on the range of distribution of the corresponding micronutrient.

Strengths of our study include the relatively large sample size, long follow-up period (up to 4 years), and a large number of measurements per subject, which provides a reasonable estimate of the within-person variability and the intraclass correlation coefficient. As eating patterns and other exposures differ for men and women, increasing the sources of dietary variability, it may be inappropriate to extrapolate our results to men. On the other hand, the multiethnic composition of the study population may have enhanced the range of dietary intakes compared with other studies. As many epidemiologic analyses are sex specific, our results are applicable to many investigations. A potential limitation of this analysis was the relatively young age composition of the study. Because older people are at a greater risk of developing chronic health problems, they are of particular interest to prospective cohort studies. Our results suggest that women 45 years and older tend to exhibit more stable plasma micronutrient levels, and hence may require fewer measurements to attain a desired precision for some micronutrients. However, due to the small proportion of older subjects in our study population (11% of the sample older than 45), a direct comparison between younger and older groups was difficult. Block et al[14] reported no particular influence of age on ICCs for circulating micronutrients.

In summary, our analysis suggests that 2 or 3 blood measurements of most carotenoids and tocopherols are enough to limit attenuation of regression coefficients to within 20% of the true value. These results are relevant to large prospective studies of the influence of micronutrients on disease with a single banked biologic specimen.

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TABLE 1

Study Sample Characteristics

No. participants		381
Total no. visits		1502
No. visits; mean (range)		4 (2–13)
Length of follow-up (days); mean (range)		452 (76–1548)
Age at first visit; mean (range)		30 (18–70)
Ethnicity; %		
	Japanese	8.9
	White	39.9
	Chinese	5.0
	Filipino	6.3
	Hawaiian/part	10.8
	Korean	1.6
	Samoan	1.0
	Other, pure	5.8
	Other, mixed	19.9
	Unknown	0.8
Tobacco smoking; %		
	Current smoker	16.0
	Current nonsmoker	84.0
Alcohol drinking; %		
	Current drinker	28.1
	Current nondrinker	71.9

TABLE 2

Descriptive Statistics, Variance Components, and Reliability of Measurements for Plasma Micronutrients

	Mean (SD) (ng/mL)	Range (ng/mL)	Median (ng/mL)	Variance[a]		Intraclass Correlation Coefficient[b]	Correlation Coefficients				Regression Coefficients			
							No. Measurements			Attenuation Factor[a]	No. Measurements			Attenuation Factor[d]
				Within	Between		5%[c]	10%[c]	20%[c]		5%[c]	10%[c]	20%[c]	
	182 (91)	22–820	167	0.0024	0.0053	0.69	5	2	1	0.83	9	4	2	0.69
	64 (28)	0–281	58	0.0020	0.0035	0.64	6	3	1	0.80	11	5	3	0.64
/	246 (116)	35–1052	230	0.0023	0.0052	0.69	4	2	1	0.83	9	4	2	0.69
	123 (48)	26–424	117	0.0024	0.0056	0.70	4	2	1	0.84	8	4	2	0.70
tein	71 (35)	1–293	66	0.0016	0.0053	0.77	3	2	1	0.88	6	3	2	0.77
n	48 (23)	1–205	44	0.0018	0.0043	0.70	4	2	1	0.84	8	4	2	0.70
	46 (17)	<1–134	43	0.0032	0.0052	0.62	6	3	1	0.79	12	6	3	0.62
nthin	163 (156)	7–1711	120	0.0015	0.0033	0.68	5	2	1	0.83	9	4	2	0.68
in	48 (34)	0–284	40	0.0021	0.0051	0.71	4	2	1	0.84	8	4	2	0.71
in	257 (198)	18–2074	207	0.0015	0.0036	0.71	4	2	1	0.84	8	4	2	0.71
	302 (124)	37–941	286	0.0052	0.0049	0.48	10	5	2	0.70	21	10	5	0.48
	233 (102)	1–756	220	0.0051	0.0041	0.45	12	6	3	0.67	24	11	5	0.45
e	69 (33)	10–646	64	0.0010	0.0007	0.43	13	6	3	0.65	26	12	6	0.43
	56 (46)	<1–437	45	0.0025	0.0045	0.64	5	3	1	0.80	11	5	3	0.64
	200 (173)	2–1599	149	0.0019	0.0054	0.74	4	2	1	0.86	7	4	2	0.74
	19 (15)	0–148	15	0.0023	0.0046	0.66	5	2	1	0.81	10	5	2	0.66
	218 (185)	0–1747	165	0.0018	0.0053	0.74	4	2	1	0.86	7	3	2	0.74
	275 (220)	3–2161	215	0.0017	0.0050	0.75	3	2	1	0.86	7	3	2	0.75
	1,322 (568)	0–4442	1233	0.0017	0.0061	0.78	3	2	1	0.88	6	3	1	0.78
	362 (261)	0–1762	283	0.0089	0.0053	0.37	16	7	3	0.61	32	15	7	0.37
rol	1,169 (717)	30–6391	1048	0.0032	0.0050	0.61	6	3	1	0.78	12	6	3	0.61
	10,587 (4,406)	3490–41,500	9524	0.0022	0.0036	0.62	6	3	1	0.79	12	6	3	0.62
	12,099 (4,327)	0–42,328	11152	0.0017	0.0023	0.57	7	4	2	0.76	15	7	3	0.57
	685 (224)	0–1981	663	0.0029	0.0032	0.53	9	4	2	0.73	17	8	4	0.53
	55 (57)	0–600	38	0.0041	0.0019	0.31	21	10	4	0.56	42	20	9	0.31

[a] Variance components given for log-transformed values.

[b] Adjusted for age, race, alcohol drinking, and tobacco smoking.

[c] Percent attenuation limited to the percent error allowed from the true value. Rounded up if more than 0.2 higher than the nearest lower integer; otherwise, rounded down.

[d] When a measurement is used.

TABLE 3

ability of Measurements for Low and High Quartiles of Plasma Micronutrient Distributions

Intraclass Correlation Coefficient[a]		Correlation Coefficients						Regression Coefficients					
		No. Measurements				Attenuation Factor[c]		No. Measurements				Attenuation Factor[c]	
		10%[b]		20%[b]				10%[b]		20%[b]			
Low Quartile	High Quartile	Low Quartile	High Quartile	Low Quartile	High Quartile	Low Quartile	High Quartile	Low Quartile	High Quartile	Low Quartile	High Quartile	Low Quartile	High Quartile
0.91	0.71	1	2	1	1	0.96	0.84	1	4	1	2	0.91	0.71
0.87	0.64	1	3	1	1	0.93	0.80	2	5	1	3	0.87	0.64
0.91	0.71	1	2	1	1	0.96	0.84	1	4	1	2	0.91	0.71
0.91	0.75	1	2	1	1	0.95	0.86	1	4	1	2	0.91	0.75
0.94	0.82	1	1	1	1	0.97	0.91	1	2	1	1	0.94	0.82
0.91	0.76	1	2	1	1	0.95	0.87	1	3	1	2	0.91	0.76
0.86	0.71	1	2	1	1	0.93	0.84	2	4	1	2	0.86	0.71
0.98	0.65	1	3	1	1	0.99	0.81	1	5	1	3	0.98	0.65
0.93	0.72	1	2	1	1	0.96	0.85	1	4	1	2	0.93	0.72
0.96	0.68	1	3	1	1	0.98	0.82	1	5	1	2	0.96	0.68
0.84	0.65	1	3	1	1	0.91	0.81	2	5	1	3	0.84	0.65
0.81	0.65	2	3	1	1	0.90	0.81	3	5	1	3	0.81	0.65
0.85	0.46	1	6	1	3	0.92	0.68	2	11	1	5	0.85	0.46
0.95	0.66	1	3	1	1	0.97	0.81	1	5	1	3	0.95	0.66
0.98	0.75	1	2	1	1	0.99	0.86	1	4	1	2	0.98	0.75
0.94	0.72	1	2	1	1	0.97	0.85	1	4	1	2	0.94	0.72
0.98	0.75	1	2	1	1	0.99	0.87	1	3	1	2	0.98	0.75
0.97	0.76	1	2	1	1	0.99	0.87	1	3	1	2	0.97	0.76
0.92	0.84	1	1	1	1	0.96	0.92	1	2	1	1	0.92	0.84
0.91	0.60	1	3	1	2	0.95	0.77	1	7	1	3	0.91	0.60
0.90	0.71	1	2	1	1	0.95	0.84	2	4	1	2	0.90	0.71
0.92	0.69	1	2	1	1	0.96	0.83	1	5	1	2	0.92	0.69
0.88	0.66	1	3	1	1	0.94	0.82	2	5	1	3	0.88	0.66
0.88	0.65	1	3	1	1	0.94	0.81	2	5	1	3	0.88	0.65
0.93	0.28	1	11	1	5	0.96	0.53	1	23	1	11	0.93	0.28

Intraclass Correlation Coefficient[a]		Correlation Coefficients						Regression Coefficients					
		No. Measurements				Attenuation Factor[c]		No. Measurements				Attenuation Factor[c]	
		10%[b]		20%[b]				10%[b]		20%[b]			
Low Quartile	High Quartile	Low Quartile	High Quartile	Low Quartile	High Quartile	Low Quartile	High Quartile	Low Quartile	High Quartile	Low Quartile	High Quartile	Low Quartile	High Quartile

lcohol drinking, and tobacco smoking.

ted to the percent error allowed from the true value. Rounded up if more than 0.2 higher than the nearest lower integer; otherwise, rounded down.

used.

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