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Intra-individual variation in one-carbon metabolism plasma biomarkers

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Abstract

Interest in the relationship between one-carbon metabolism (OCM) and carcinogenesis is intensifying, leading to increased use of related biomarkers as measures of exposure. Little is known, however, about intra-individual variation in these markers and if use of a single measure is appropriate for assessing exposure-disease relationships in epidemiologic studies. We evaluated intra-individual variation in plasma concentrations of 19 OCM biomarkers in a sample of 147 African American and 68 non-Hispanic white participants from the Southern Community Cohort Study who donated blood samples and responded to questionnaires at two points in time from 2005–2008. Weighted kappa coefficients (κ) were calculated to assess agreement between quartile assignments based on the repeated measures. Adjusted intra-class correlation coefficients (ICCs) were also used to assess the consistency of the two measurements. Most (16 of 19) OCM biomarkers showed moderate or better agreement for quartile assignment at the two timepoints, with only methionine, methionine sulfoxide, and cystathionine having $\kappa < 0.40$. The median adjusted ICC across the 19 biomarkers was 0.60. Reproducibility was highest for flavin mononucleotide (ICC=0.84, 95% confidence interval (CI) 0.79–0.87) and lowest for methionine and its oxidative product methionine sulfoxide (ICC=0.22, 95%CI 0.09–0.34; ICC=0.20, 95%CI 0.07–0.32, respectively). Overall, intra-individual variation in OCM biomarkers was similar for African Americans and whites, and for males and females. Our results suggest that, with the

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exception of methionine and methionine sulfoxide, OCM biomarkers generally have good intra-individual reproducibility and can be considered reliable exposure measures in epidemiologic studies.

Keywords

One-carbon metabolism; intra-class correlation; biological markers; prospective studies; reproducibility of results

INTRODUCTION

Scientific interest in one-carbon metabolism (OCM) and cancer risk is increasing. OCM denotes a metabolic system made up of interdependent pathways that involve the transfer of one-carbon units for numerous biosynthetic reactions relevant to DNA synthesis, repair, and methylation (1). The primary component of the system is folate, and the transfer reactions involve conversions between several of its forms. Other key components include vitamins B₂, B₆, and B₁₂, each of which acts as an essential cofactor for one or more enzymes that catalyze one-carbon transfer reactions (2). Because an important role of OCM is to provide the methyl groups needed for DNA synthesis and methylation, imbalances in the system are hypothesized to lead to genomic instability and subsequent changes in the expression of oncogenes or tumor suppressor genes (3,4).

The current availability of laboratory measurement methods for a large suite of OCM biomarkers has enabled them to be increasingly used as objective measures of exposure in epidemiologic research. A number of studies have already reported on associations between OCM biomarkers and risk of adult solid tumors (5–11). However, there has been limited assessment of the within-person stability of these markers over time. This issue is important for prospective studies whose samples are collected once at baseline, with measurements assumed to represent individuals' typical levels. If this assumption is violated, the biomarker will not serve to reflect the exposure of interest and resultant relative risk estimates could be biased (12). Thus, the purpose of this study was to assess intra-individual variation in 19 OCM biomarkers among African American and white participants from the prospective Southern Community Cohort Study (SCCS).

MATERIALS AND METHODS

Study population

Methods for the establishment of the SCCS cohort and baseline data collection have been described in detail elsewhere (13). Briefly, from 2002–2009, over 85,000 participants were enrolled in the SCCS throughout 12 states in the southeastern US. Eligibility was restricted to those aged 40–79 who spoke English and had not undergone treatment for cancer within the past year. The vast majority of participants self-reported their race as either African American (65%) or non-Hispanic white (30%). Approximately half of the participants provided a non-fasting, 10 mL venous blood sample (using an EDTA plasma tube) at the time of their in-person baseline interview. These participants, all of whom enrolled at a

community health center (CHC), serve as the study base for this analysis. Blood samples were kept in standard refrigeration at the CHCs and then shipped the same day in ice packs for overnight delivery to Vanderbilt University where they were separated into components and frozen at -80°C the same day. These samples spent minimal time at room temperature, thus limiting the possibility for degradation or accumulation of the measured markers (14).

During May–October 2008, we attempted to contact all ($N=1,102$) SCCS participants from our study base who enrolled at one of nine active CHC enrollment sites either 12, 24, or 36 months prior. Invitations were mailed asking participants to return to the CHC and provide a second blood sample. Approximately 66% ($N=662$) of those assumed to have received the invitation ($N=1,010$; 92 invitations were returned as undeliverable) consented to provide a repeat blood sample and complete a brief interview targeting potential changes in lifestyle factors. Blood samples were collected and handled using the same procedures as at baseline. The distribution of time since enrollment for this group ($N=472$ one year prior, $N=152$ two years prior, and $N=38$ three years prior) reflects the fact that CHCs were typically only active as SCCS enrollment sites for 1–2 years. For the present analysis we selected a random sample of 100 participants whose blood samples were spaced one year apart, 100 spaced two years apart, and all ($N=38$) spaced three years apart.

The SCCS was approved by Institutional Review Boards at Vanderbilt University and Meharry Medical College. All subjects provided written informed consent for the main study and separately for the collection of the second blood sample.

Biomarker measurement

Measurement of plasma levels of 19 OCM biomarkers was conducted at Bevitall A/S (Bergen, Norway) by use of published methods. Folate and cobalamin were analyzed by microbiological assays (15,16). Methionine, glycine, serine, total homocysteine, total cysteine, and methylmalonic acid were analyzed using a gas chromatography–mass spectrometry based method (17). The remaining analytes (pyridoxal, pyridoxal 5'-phosphate, pyridoxic acid, riboflavin, flavin mononucleotide, tryptophan, methionine sulfoxide, betaine, choline, dimethylglycine, and cystathionine) were measured using liquid chromatography–tandem mass spectrometry based assays (18,19). Plasma from the baseline and repeat samples for each subject were analyzed within the same batch. The average intra-assay coefficients of variation calculated from eight blinded triplicate sets of identical QC samples were as follows: tryptophan 1.5%; methionine 1.6%; serine 1.9%; total homocysteine 2.0%; total cysteine 2.1%; glycine 2.3%; choline 2.6%; cobalamin 2.9%; betaine 3.4%; methylmalonic acid 5.1%; folate 5.5%; pyridoxal 5'-phosphate 7.1%; pyridoxal 8.2%; cystathionine 8.9%; dimethylglycine 9.3%; pyridoxic acid 10.4%; methionine sulfoxide 10.7%; riboflavin 16.7%; flavin mononucleotide 18.0%.

Statistical analysis

To assess the level of agreement between baseline and repeat sample measurements, we categorized analyte measurements according to their quartile positions at each of the two time points and then calculated weighted kappa (κ) coefficients. Quartile classifications varied across subgroup analyses (e.g., κ values for men were based on male-specific

quartiles). Kappa values were interpreted according to the criteria of Landis and Koch (20), with agreement classified as slight to fair for values 0.01–0.40, moderate for 0.41–0.60, substantial for 0.61–0.80, and almost perfect for >0.80.

Adjusted intra-class correlation coefficients (ICC) were calculated using a random effects mixed model with log-transformed analyte values to improve normality. ICCs were adjusted for the following covariates measured at both time periods: age (continuous), race, sex, body mass index (BMI) (<25.0 / 25.0–29.9 / 30.0 kg/m²), smoking status (current vs. not), folic acid-containing supplement use including multivitamins (yes/no), season of blood collection (spring/summer/fall), time of sample collection (8:00am – 11:59am/12:00pm – 3:59pm/ 4:00pm or later), number of hours since the last meal before sample collection (continuous), and number of years between samples (1/2/3). Analyses were performed using SAS/STAT software, version 9.3 (SAS Institute, Inc., Cary, NC).

RESULTS

Eleven of the samples were not assayed and 12 had suffered hemolysis, leaving 215 of the 238 samples (90%) available for analysis. These included 97 participants with repeat samples drawn one year post-baseline, 85 drawn two years post-baseline, and 33 drawn three years post-baseline. All samples collected three years post-baseline were from African American participants. Overall, 68% of the included participants were African American and 56% were female. Table 1 presents descriptive characteristics of the study group at baseline and at the time of the repeat sample, as well as OCM biomarker measurements from both timepoints. Across all study participants, BMI, time of day of blood sampling, and smoking status were similar ($p>0.05$) at the two timepoints. Season of blood collection varied across timepoints ($p<0.001$), although seasonal categories masked very good calendar time correspondence between the blood samples as measured in days. For example, if a one day difference in calendar time represents an individual providing a blood sample on June 13th one year and on June 14th the next year, the median and interquartile range for calendar days apart for our samples were 13 (5–22) days. Self-report of folic acid-containing supplement use decreased in the time between sampling ($p<0.001$).

Overall, the proportion of participants classified in the exact same quartile at both time points ranged from 34.0 to 59.5% depending on the biomarker. Across the 19 biomarkers, weighted κ values ranged from ~0.30 for methionine and its oxidative product methionine sulfoxide to 0.72 for cobalamin (Table 2). When assessed by sex, more than half (12/19) of the analytes had the same κ classification (see Materials and Methods) for men and women, whereas 5 had stronger agreement for men and 2 had stronger agreement for women. With regard to race, 10 of the 19 analytes had the same κ classification for African Americans and whites, whereas 5 had stronger agreement for whites and 4 had stronger agreement for African Americans. Stratification by number of years between samples (not shown) resulted in κ values ranging from 0.27 (methionine sulfoxide) to 0.79 (methylmalonic acid) for the one-year group, 0.18 (methionine) to 0.70 (glycine) for the two-year group, and 0.10 (methionine sulfoxide) to 0.79 (glycine) for the three-year group (and median κ values across analytes of 0.57, 0.55, and 0.55 across years 1–3, respectively). The only analyte exhibiting a substantial difference in κ value across years was methylmalonic acid, which

had slight to fair agreement for samples spaced three years apart ($\kappa=0.33$; 95%CI 0.003–0.66) and substantial agreement for samples spaced two-years apart ($\kappa=0.68$; 95%CI 0.55–0.81) and one-year apart ($\kappa=0.79$; 95%CI 0.70–0.87).

Overall adjusted ICCs ranged from ~0.20 (methionine and methionine sulfoxide) to 0.84 (flavin mononucleotide), with a median ICC across analytes of 0.60 (Table 3). ICCs for each analyte were generally similar by sex, with median ICC values of 0.55 for both men and women. Cystathionine showed the largest difference by sex (ICC=0.62 for men; ICC=0.29 for women). By race, ICCs were higher among whites for 11 of the 19 analytes, and the median ICC value was higher for whites (0.61) than African Americans (0.52). We also stratified by sample spacing in years (not shown) and found that the median ICC across all analytes was highest for those spaced one year apart (0.63), although it was only slightly lower for those spaced two (0.52) and three years apart (0.51). For individual analytes there was no discernible pattern in ICC values across year of sample spacing, nor across strata of participant age (<55 vs. 55+ years, not shown).

DISCUSSION

To our knowledge, this is the first detailed report of intra-individual variation for this set of OCM biomarkers. Analysis of plasma samples taken one to three years apart showed good within-person reproducibility; three-quarters of the analytes had ICCs 0.47 and more than half had ICCs 0.60. Stratified analyses revealed some differences in our findings across race and sex groups, but no strong patterns emerged. For the most part, analytes performed similarly for males and females. Slightly more variability was observed when comparing across race. Aside from chance, a possible explanation is the greater change in use of folic acid-containing supplements between baseline and repeat blood sampling for African Americans (from 40% to 20% during this time period) than whites (from 49% to 41%). ICCs were adjusted for supplement use at both timepoints, but in ancillary analyses excluding all folic acid-containing supplement users (not shown), ICCs were not appreciably better in one racial group vs. the other.

Within-person reproducibility appeared reasonable for our samples spaced one, two, and even three years apart. However, ICC results for samples spaced three years apart were based on a small number of participants, had 6 of the 19 confidence intervals including the null value, and thus should be interpreted with caution. Further investigation with larger sample sizes is needed to assess the time point at which baseline samples fail to be predictive of marker levels over the long term.

A study from the Dutch arm of the European Prospective Investigation into Cancer and Nutrition (EPIC) recently reported on reproducibility for four OCM biomarkers in serum samples taken 2–5 years apart: folate, vitamins B6 and B12, and homocysteine (21). Our overall adjusted ICCs were similar to theirs for folate (0.47 SCCS, 0.45 EPIC), slightly lower for vitamin B12/cobalamin (0.61 SCCS, 0.75 EPIC), higher for vitamin B6/pyridoxal 5'-phosphate (0.60 SCCS, 0.38 EPIC), and lower for homocysteine (0.47 SCCS, 0.91 EPIC) (21). In general, we found poorer reproducibility for homocysteine than several previous studies (21–25). For some of these studies the time elapsed between samples was

substantially shorter, fasting samples were used, and the ICCs presented were crude (22–24). Our estimates were adjusted for several factors which we generally found reduced the ICCs (i.e., our unadjusted ICC for homocysteine was 0.63 (95% CI 0.54–0.70)).

This study has several strengths. It is the first examination of intra-individual variation for these 19 OCM biomarkers and was conducted in a diverse study population that allowed comparisons by sex and race. Blood was collected in EDTA tubes, a method that confers enhanced stability for most of these biomarkers during the sample handling and processing period (14). Finally, due to repeated questionnaires, we were able to present ICCs adjusted for a number of potentially influential factors including vitamin supplement use, smoking, and number of hours since the participant's last meal. Our results in summary suggest that, with the exception of methionine and methionine sulfoxide, OCM biomarkers generally have good within-person reproducibility and are sufficiently reliable for use in epidemiologic studies.

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Table 1

Characteristics of 215 SCCS participants with baseline and repeat plasma samples.

Characteristic	Baseline sample (2005–2007)	Repeat sample (2008)
Mean (SD) age, yrs	54.7 (8.6)	57.0 (8.6)
Mean (SD) BMI, kg/m ²	31.7 (8.2)	32.0 (7.9)
Mean (SD) time since last meal at blood sampling, hrs	9.2 (6.6)	7.2 (5.5)
Season of sample collection, N (%)		
Spring (March/April/May)	61 (28.4%)	44 (20.5%)
Summer (June/July/August)	139 (64.7%)	136 (63.3%)
Fall (September/October/November)	15 (7.0%)	35 (16.3%)
Time of sample collection, N (%)		
Morning (8am – 11:59am)	91 (42.3%)	95 (44.2%)
Early Afternoon (12:00pm – 3:59pm)	67 (31.2%)	64 (29.8%)
Late Afternoon (4pm or later)	57 (26.5%)	56 (26.0%)
Current smoker, N (%)	72 (33.5%)	77 (35.8%)
Folic acid-containing supplement user ^a , N (%)	91 (42.3%)	57 (26.5%)
OCM biomarker measurements, Median (Q1, Q3)		
Riboflavin (vitamin B ₂ , nmol/L)	15.8 (9.0, 27.5)	15.0 (9.1, 27.7)
Flavin Mononucleotide (nmol/L)	14.4 (11.0, 19.5)	14.7 (11.6, 21.6)
Pyridoxal (nmol/L)	5.3 (3.8, 8.1)	6.1 (4.1, 10.0)
Pyridoxal 5'-phosphate (nmol/L)	51.6 (37.0, 81.2)	52.2 (34.7, 91.8)
Pyridoxic Acid (nmol/L)	22.2 (13.9, 40.4)	21.0 (14.5, 41.1)
Cobalamin (vitamin B ₁₂ , pmol/L)	437.8 (344.7, 534.8)	432.8 (331.0, 518.4)
Folate (nmol/L)	13.3 (8.9, 18.3)	13.2 (9.0, 19.3)
Methylmalonic Acid (μmol/L)	0.17 (0.14, 0.22)	0.19 (0.15, 0.24)
Total Homocysteine (μmol/L)	15.8 (13.2, 18.9)	15.1 (12.2, 18.1)
Total Cysteine (μmol/L)	293.4 (266.2, 327.5)	295.5 (272.8, 330.7)
Glycine (μmol/L)	248.9 (204.2, 304.1)	258.1 (216.4, 321.0)
Methionine (μmol/L)	24.5 (20.2, 28.5)	25.3 (21.6, 31.0)
Methionine Sulfoxide (μmol/L)	0.9 (0.7, 1.2)	1.1 (0.8, 1.5)
Serine (μmol/L)	111.5 (93.6, 126.5)	110.4 (97.0, 127.1)
Betaine (μmol/L)	39.1 (32.0, 47.7)	41.0 (34.0, 49.7)
Choline (μmol/L)	12.6 (10.6, 15.1)	12.4 (10.4, 15.1)
Dimethylglycine (μmol/L)	3.8 (3.0, 4.9)	4.1 (3.1, 5.2)
Cystathionine (μmol/L)	0.18 (0.13, 0.25)	0.21 (0.15, 0.32)
Tryptophan (μmol/L)	54.0 (47.4, 63.1)	56.5 (47.5, 64.4)

Abbreviations: SCCS: Southern Community Cohort Study; OCM: one-carbon metabolism; BMI: body mass index; Q1: quartile 1 cutoff; Q3: quartile 3 cutoff

^a includes folic acid supplements and multivitamin supplements

Table 2

Weighted kappa (κ) values with 95% confidence intervals (CI) for quartile agreement between baseline and repeat measurements of one-carbon metabolism (OCM) biomarkers, overall and stratified by sex and race.

OCM Biomarker	Weighted κ (95% CI)			
	Overall (N = 215)	Male ^a (N = 94)	Female ^a (N = 121)	African American ^b (N = 147)
Riboflavin	0.61 (0.50, 0.71)	0.69 (0.57, 0.81)	0.58 (0.44, 0.72)	0.70 (0.60, 0.80)
Flavin Mononucleotide	0.56 (0.46, 0.66)	0.70 (0.60, 0.81)	0.50 (0.35, 0.65)	0.62 (0.51, 0.73)
Pyridoxal	0.53 (0.42, 0.63)	0.65 (0.52, 0.78)	0.46 (0.32, 0.61)	0.50 (0.37, 0.63)
Pyridoxal 5'-phosphate	0.62 (0.53, 0.71)	0.58 (0.42, 0.73)	0.64 (0.53, 0.75)	0.53 (0.41, 0.65)
Pyridoxic Acid	0.51 (0.40, 0.62)	0.51 (0.36, 0.67)	0.51 (0.36, 0.66)	0.41 (0.26, 0.55)
Cobalamin	0.72 (0.63, 0.80)	0.71 (0.59, 0.83)	0.69 (0.58, 0.80)	0.72 (0.61, 0.82)
Folate	0.51 (0.40, 0.62)	0.44 (0.26, 0.61)	0.51 (0.37, 0.64)	0.49 (0.36, 0.61)
Methylmalonic Acid	0.71 (0.63, 0.79)	0.69 (0.55, 0.82)	0.75 (0.65, 0.85)	0.67 (0.57, 0.77)
Total Homocysteine	0.59 (0.50, 0.69)	0.50 (0.34, 0.65)	0.55 (0.42, 0.67)	0.62 (0.50, 0.73)
Total Cysteine	0.62 (0.53, 0.71)	0.51 (0.34, 0.67)	0.72 (0.63, 0.81)	0.61 (0.50, 0.72)
Glycine	0.71 (0.64, 0.78)	0.73 (0.63, 0.83)	0.72 (0.62, 0.83)	0.66 (0.56, 0.76)
Methionine	0.31 (0.19, 0.43)	0.29 (0.09, 0.48)	0.28 (0.12, 0.44)	0.32 (0.17, 0.46)
Methionine Sulfoxide	0.30 (0.17, 0.42)	0.26 (0.06, 0.45)	0.39 (0.23, 0.54)	0.25 (0.09, 0.40)
Serine	0.61 (0.52, 0.69)	0.61 (0.48, 0.74)	0.53 (0.48, 0.70)	0.58 (0.47, 0.69)
Betaine	0.65 (0.57, 0.74)	0.65 (0.54, 0.76)	0.62 (0.51, 0.73)	0.67 (0.57, 0.76)
Choline	0.51 (0.40, 0.62)	0.45 (0.28, 0.63)	0.51 (0.37, 0.66)	0.47 (0.32, 0.61)
Dimethylglycine	0.71 (0.64, 0.79)	0.66 (0.53, 0.79)	0.64 (0.52, 0.76)	0.70 (0.61, 0.80)
Cystathionine	0.35 (0.22, 0.47)	0.39 (0.21, 0.57)	0.32 (0.15, 0.49)	0.29 (0.14, 0.45)
Tryptophan	0.44 (0.32, 0.55)	0.48 (0.32, 0.65)	0.30 (0.12, 0.47)	0.49 (0.35, 0.62)

Abbreviations: SCCS: Southern Community Cohort Study; OCM: one-carbon metabolism; CI: confidence interval

^a κ values are based on sex-specific quartile cutpoints

^b κ values are based on race-specific quartile cutpoints

Table 3

Adjusted intra-class correlation coefficients (ICC) for baseline and repeat measurements of one-carbon metabolism (OCM) biomarkers, overall and stratified by sex and race.

OCM Biomarker	Adjusted ICC ^a (95% CI)				
	Overall (N = 215)	Male (N = 94)	Female (N = 121)	African American (N = 147)	White (N = 68)
Riboflavin	0.75 (0.69, 0.81)	0.86 (0.80, 0.91)	0.62 (0.50, 0.72)	0.85 (0.80, 0.89)	0.51 (0.31, 0.66)
Flavin Mononucleotide	0.84 (0.79, 0.87)	0.84 (0.76, 0.89)	0.82 (0.75, 0.87)	0.87 (0.82, 0.90)	0.61 (0.44, 0.74)
Pyridoxal	0.31 (0.18, 0.42)	0.17 (−0.04, 0.36)	0.28 (0.11, 0.44)	0.10 (−0.06, 0.26)	0.43 (0.22, 0.61)
Pyridoxal 5'-phosphate	0.60 (0.51, 0.68)	0.44 (0.26, 0.58)	0.65 (0.54, 0.74)	0.49 (0.36, 0.61)	0.73 (0.60, 0.82)
Pyridoxic Acid	0.36 (0.23, 0.47)	0.26 (0.06, 0.44)	0.35 (0.19, 0.50)	0.18 (0.02, 0.33)	0.54 (0.36, 0.69)
Cobalamin	0.61 (0.52, 0.69)	0.60 (0.46, 0.72)	0.55 (0.41, 0.66)	0.52 (0.39, 0.63)	0.82 (0.72, 0.88)
Folate	0.47 (0.36, 0.57)	0.32 (0.13, 0.49)	0.52 (0.37, 0.64)	0.47 (0.34, 0.59)	0.28 (0.05, 0.49)
Methylmalonic Acid	0.82 (0.77, 0.86)	0.83 (0.76, 0.88)	0.79 (0.72, 0.85)	0.75 (0.68, 0.82)	0.88 (0.81, 0.92)
Total Homocysteine	0.47 (0.36, 0.57)	0.42 (0.24, 0.58)	0.48 (0.33, 0.60)	0.55 (0.42, 0.65)	0.20 (−0.04, 0.41)
Total Cysteine	0.62 (0.53, 0.69)	0.53 (0.37, 0.66)	0.67 (0.55, 0.75)	0.60 (0.49, 0.70)	0.69 (0.55, 0.80)
Glycine	0.75 (0.68, 0.80)	0.72 (0.60, 0.80)	0.75 (0.67, 0.82)	0.69 (0.59, 0.77)	0.83 (0.73, 0.89)
Methionine	0.22 (0.09, 0.34)	0.29 (0.10, 0.47)	0.09 (−0.09, 0.26)	0.30 (0.15, 0.44)	0.00 (−0.23, 0.23)
Methionine Sulfoxide	0.20 (0.07, 0.32)	0.11 (−0.09, 0.30)	0.24 (0.07, 0.40)	0.17 (0.01, 0.33)	0.20 (−0.04, 0.42)
Serine	0.66 (0.57, 0.73)	0.62 (0.48, 0.73)	0.65 (0.53, 0.74)	0.65 (0.55, 0.74)	0.62 (0.45, 0.74)
Betaine	0.66 (0.58, 0.73)	0.74 (0.64, 0.82)	0.59 (0.46, 0.69)	0.64 (0.53, 0.72)	0.68 (0.53, 0.79)
Choline	0.54 (0.44, 0.63)	0.54 (0.38, 0.64)	0.52 (0.38, 0.64)	0.51 (0.38, 0.62)	0.55 (0.36, 0.69)
Dimethylglycine	0.75 (0.69, 0.81)	0.76 (0.66, 0.83)	0.73 (0.63, 0.80)	0.76 (0.68, 0.82)	0.74 (0.61, 0.83)
Cystathionine	0.52 (0.41, 0.61)	0.62 (0.48, 0.73)	0.29 (0.12, 0.45)	0.34 (0.18, 0.47)	0.67 (0.51, 0.78)
Tryptophan	0.39 (0.28, 0.50)	0.55 (0.39, 0.68)	0.32 (0.15, 0.47)	0.51 (0.38, 0.62)	0.10 (−0.13, 0.33)

Abbreviations: OCM: one-carbon metabolism; ICC: intra-class correlation coefficient; CI: confidence interval

^a Adjusted for: age, race (overall and sex-stratified results only), sex (overall and race-stratified results only), body mass index, current smoking status, folic acid containing supplement use, season of sample collection, time of day of sample collection, hours since last meal before sample collection, and years between baseline and repeat sample.