Published OnlineFirst August 15, 2013; DOI: 10.1158/1055-9965.EPI-13-0420

Cancer Epidemiology, Biomarkers & Prevention

Intraindividual Variation in One-Carbon Metabolism Plasma Biomarkers

Elizabeth L. Cope, Martha J. Shrubsole, Sarah S. Cohen, et al.

Cancer Epidemiol Biomarkers Prev 2013;22:1894-1899. Published OnlineFirst August 15, 2013.

Short Communication

Cancer Epidemiology, Biomarkers & Prevention

Intraindividual Variation in One-Carbon Metabolism Plasma Biomarkers

Elizabeth L. Cope[1], Martha J. Shrubsole[2,3], Sarah S. Cohen[1], Qiuyin Cai[2,3], Jie Wu[2], Per Magne Ueland[4,5], Øivind Midttun[6], Jennifer S. Sonderman[1], William J. Blot[1,2,3], and Lisa B. Signorello[7,8]

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 the intraindividual variation in these markers and whether or not the use of a single measure is appropriate for assessing exposure–disease relationships in epidemiologic studies. We evaluated the intraindividual 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 time points from 2005 to 2008. Weighted kappa coefficients (κ) were calculated to assess the agreement between quartile assignments based on the repeated measures. Adjusted intraclass correlation coefficients (ICC) were also used to assess the consistency of the two measurements. Most (16/19) OCM biomarkers showed a moderate or better agreement for quartile assignment at the two time points, with only methionine, methionine sulfoxide, and cystathionine having $\kappa \leq 0.40$. 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, the intraindividual variation in OCM biomarkers was similar for African Americans and whites and for males and females. Our results suggest that with the exception of methionine and methionine sulfoxide, OCM biomarkers generally have good intraindividual reproducibility and can be considered as reliable exposure measures in epidemiologic studies. *Cancer Epidemiol Biomarkers Prev; 22 (10); 1894–9. ©2013 AACR.*

Introduction

The 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 B_2, B_6, and B_{12}, 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 the 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 the intraindividual variation in 19 OCM biomarkers among African American and white participants from the prospective Southern Community Cohort Study (SCCS).

Authors' Affiliations: [1]International Epidemiology Institute, Rockville, Maryland; [2]Division of Epidemiology, Department of Medicine; [3]Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee; [4]Section for Pharmacology, Institute of Medicine, University of Bergen; [5]Laboratory of Clinical Biochemistry, Haukeland University Hospital; [6]BEVITAL, Bergen, Norway; [7]Department of Epidemiology, Harvard School of Public Health; and [8]Dana-Farber/Harvard Cancer Center, Boston, Massachusetts

Corresponding Author: Lisa B. Signorello, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115. Phone: 617-432-2226; Fax: 617-566-7805; E-mail: Signorello@hsph.harvard.edu

doi: 10.1158/1055-9965.EPI-13-0420

Materials and Methods

Study population

Methods for the establishment of the SCCS and baseline data collection have been described in detail elsewhere (13). Briefly, from 2002 to 2009, more than 85,000 participants were enrolled in the SCCS throughout 12 states in the southeastern U.S. 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 (Nashville, TN), 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 to 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 to 2 years. For the present analysis, we selected a random sample of 100 participants whose blood samples were spaced 1 year apart, 100 spaced 2 years apart, and all ($N = 38$) spaced 3 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

The measurement of plasma levels of 19 OCM biomarkers was conducted at Bevital A/S by the use of published methods. Folate and cobalamin were analyzed by microbiologic 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 quality control 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%; and 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 the 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 intraclass 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/m^2), 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 (8:00 am–11:59 am/12:00 pm–3:59 pm/4:00 pm or later), number of hours since the last meal before sample collection (continuous), and number of years between samples (1/2/3). Analyses were conducted using SAS/STAT software, version 9.3 (SAS Institute, Inc.).

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 1 year post-baseline, 85 drawn 2 years post-baseline, and 33 drawn 3 years post-baseline. All samples collected 3 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 time points. Across all study participants, BMI, time of day of blood sampling, and smoking status were similar

Table 1. Characteristics of 215 SCCS participants with baseline and repeat plasma samples

Characteristic	Baseline sample (2005–2007)	Repeat sample (2008)
Mean (SD) age, y	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, h	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 (8 am–11:59 am)	91 (42.3%)	95 (44.2%)
Early afternoon (12:00 pm–3:59 pm)	67 (31.2%)	64 (29.8%)
Late afternoon (4 pm 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)

BMI, body mass index; Q1, quartile 1 cutoff; Q3, quartile 3 cutoff.
[a]Includes folic acid supplements and multivitamin supplements.

($P > 0.05$) at the two time points. Season of blood collection varied across time points ($P < 0.001$), 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 the calendar time represents an individual providing a blood sample on June 13 one year and on June 14 the next year, the median and interquartile ranges for calendar days apart for our samples were 13 (5–22) days. The 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 timepoints ranged from 34.0%–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 a stronger agreement for men and 2 had a 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 a stronger agreement for whites and 4 had a 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 1-year group, 0.18 (methionine) to 0.70 (glycine) for the 2-year group, and 0.10 (methionine sulfoxide) to 0.79 (glycine) for the 3-year

Table 2. Weighted kappa (κ) values with 95% CI for quartile agreement between baseline and repeat measurements of OCM biomarkers, overall and stratified by sex and race

	Weighted κ (95% CI)				
OCM biomarker	Overall ($N = 215$)	Male[a] ($N = 94$)	Female[a] ($N = 121$)	African American[b] ($N = 147$)	White[b] ($N = 68$)
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)	0.40 (0.17–0.63)
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)	0.47 (0.27–0.67)
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)	0.62 (0.46–0.79)
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)	0.71 (0.57–0.84)
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)	0.64 (0.49–0.78)
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)	0.71 (0.56–0.87)
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)	0.48 (0.27–0.69)
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)	0.69 (0.56–0.82)
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)	0.58 (0.40–0.75)
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)	0.66 (0.53–0.79)
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)	0.82 (0.75–0.90)
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)	0.13 (–0.12–0.37)
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)	0.34 (0.12–0.56)
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)	0.65 (0.50–0.79)
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)	0.70 (0.56–0.84)
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)	0.60 (0.44–0.75)
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)	0.70 (0.57–0.82)
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)	0.39 (0.19–0.60)
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)	0.29 (0.08–0.51)

[a]κ values are based on sex-specific quartile cutpoints.
[b]κ values are based on race-specific quartile cutpoints.

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 the κ value across years was methylmalonic acid, which had a slight to fair agreement for samples spaced 3 years apart [$\kappa = 0.33$; 95% confidence interval (CI) 0.003–0.66] and substantial agreement for samples spaced 2 years apart ($\kappa = 0.68$; 95% CI 0.55–0.81) and 1 year apart ($\kappa = 0.79$; 95% CI 0.70–0.87).

Overall adjusted ICCs ranged from approximately 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 2 (0.52) and 3 years apart (0.51). For individual analytes, there was no discernible pattern in ICC values across the 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 intraindividual variation for this set of OCM biomarkers. Analysis of plasma samples taken 1 to 3 years apart showed good within-person reproducibility; three quarters of the analytes had ICCs $\geq$0.47 and more than half had ICCs $\geq$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 time points, but in ancillary analyses excluding all folic acid-containing supplement users (not shown), ICCs were not appreciably better in one racial group versus the other.

The within-person reproducibility seemed reasonable for our samples spaced 1, 2, and even 3 years apart. However, ICC results for samples spaced 3 years apart were based on a small number of participants, had 6 of the 19 CIs 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

Table 3. Adjusted ICCs for baseline and repeat measurements of OCM biomarkers, overall and stratified by sex and race

	Adjusted ICC[a] (95% CI)				
OCM biomarker	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)

[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.

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 to 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; ref. 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 intraindividual 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 the 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: E.L. Cope, J.S. Sonderman, L.B. Signorello
Development of methodology: E.L. Cope, P.M. Ueland, J.S. Sonderman
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.S. Cohen, Q. Cai, J. Wu, P.M. Ueland, Ø. Midttun, W.J. Blot, L.B. Signorello
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E.L. Cope, M.J. Shrubsole, S.S. Cohen, J. Wu, J.S. Sonderman, L.B. Signorello
Writing, review, and/or revision of the manuscript: E.L. Cope, M.J. Shrubsole, S.S. Cohen, Q. Cai, P.M. Ueland, Ø. Midttun, J.S. Sonderman, W.J. Blot, L.B. Signorello
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.S. Cohen, J. Wu, P.M. Ueland, J.S. Sonderman
Study supervision: L.B. Signorello

Acknowledgments

The authors thank Regina Courtney for her excellent laboratory assistance.

Grant Support

The SCCS is funded by grant number R01 CA092447 from the NIH/National Cancer Institute. Sample preparation was conducted at the Survey and Biospecimen Shared Resource that is supported in part by the Vanderbilt-Ingram Cancer Center (P30CA068485).

Received April 23, 2013; revised July 15, 2013; accepted August 2, 2013; published OnlineFirst August 15, 2013.

References

1. Stover PJ. Physiology of folate and vitamin B12 in health and disease. Nutr Rev 2004;62:S3–13.
2. Friso S, Choi SW. Gene-nutrient interactions in one-carbon metabolism. Curr Drug Metab 2005;6:37–46.
3. Kim YI. Folate and carcinogenesis: evidence, mechanisms, and implications. J Nutr Biochem 1999;10:66–88.
4. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr 2000;130:129–32.
5. Weinstein SJ, Albanes D, Selhub J, Graubard B, Lim U, Taylor PR, et al. One-carbon metabolism biomarkers and risk of colon and rectal cancers. Cancer Epidemiol Biomarkers Prev 2008;17:3233–40.
6. Le Marchand L, White KK, Nomura AM, Wilkens LR, Selhub JS, Tiirikainen M, et al. Plasma levels of B vitamins and colorectal cancer risk: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2009;18:2195–201.
7. Eussen SJ, Vollset SE, Hustad S, Midttun Ø, Meyer K, Fredriksen A, et al. Plasma vitamins B2, B6, and B12, and related genetic variants as predictors of colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2010;19:2549–61.
8. Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, et al. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. J Natl Cancer Inst 2003;95:373–80.
9. Schernhammer E, Wolpin B, Rifai N, Cochrane B, Manson JA, Ma J, et al. Plasma folate, vitamin B6, vitamin B12, and homocysteine and pancreatic cancer risk in four large cohorts. Cancer Res 2007;67:5553–60.
10. Johansson M, Van Guelpen B, Vollset SE, Hultdin J, Bergh A, Key T, et al. One-carbon metabolism and prostate cancer risk: prospective investigation of seven circulating B vitamins and metabolites. Cancer Epidemiol Biomarkers Prev 2009;18:1538–43.
11. Gibson TM, Weinstein SJ, Mayne ST, Pfeiffer RM, Selhub J, Taylor PR, et al. A prospective study of one-carbon metabolism biomarkers and risk of renal cell carcinoma. Cancer Causes Control 2010;21:1061–9.
12. White E. Measurement error in biomarkers. IARC Sci Publ 2011;163:143–61.
13. Signorello LB, Hargreaves MK, Steinwandel MD, Zheng W, Cai Q, Schlundt DG, et al. Southern Community Cohort Study: establishing a cohort to investigate health disparities. J Natl Med Assoc 2005;97:972–9.
14. Hustad S, Eussen S, Midttun O, Ulvik A, van de Kant PM, Morkrid L, et al. Kinetic modeling of storage effects on biomarkers related to B vitamin status and one-carbon metabolism. Clin Chem 2012;58:402–10.
15. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol 1997;281:43–53.
16. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. J Clin Pathol 1991;44:592–5.
17. Windelberg A, Årseth O, Kvalheim G, Ueland PM. Automated assay for the determination of methylmalonic acid, total homocysteine, and related amino acids in human serum or plasma by means of methylchloroformate derivatization and gas chromatography-mass spectrometry. Clin Chem 2005;51:2103–9.
18. Midttun Ø, Kvalheim G, Ueland PM. High-throughput, low-volume, multianalyte quantification of plasma metabolites related to one-carbon metabolism using HPLC-MS/MS.Anal Bioanal Chem 2013;405:2009–17.
19. Midttun Ø, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2009;23:1371–9.
20. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159–74.
21. Leenders M, Ros MM, Sluijs I, Boshuizen HC, van Gils CH, Jansen EHJM, et al. Reliability of selected antioxidants and compounds involved in one-carbon metabolism in two Dutch cohorts. Nutr Cancer 2013;65:17–24.
22. Garg UC, Zheng ZJ, Folsom AR, Moyer YS, Tsai MY, McGovern P, et al. Short-term and long-term variability of plasma homocysteine measurement. Clin Chem 1997;43:141–5.
23. McKinley MC, Strain JJ, McPartlin J, Scott JM, McNulty H. Plasma homocysteine is not subject to seasonal variation. Clin Chem 2001;47:1430–6.
24. Tsai MY, McGovern P, Kennedy EL, Hanson NQ. Short-term variability in the measurement of plasma homocysteine, fasting and post-methionine loading. Clin Biochem 2001;34:49–52.
25. Clarke R, Woodhouse P, Ulvik A, Frost C, Sherliker P, Refsum H, et al. Variability and determinants of total homocysteine concentrations in plasma in an elderly population. Clin Chem 1998;44:102–7.