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Comparison of Two Dietary Folate Intake Instruments and Their Validation by RBC Folate

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An optimal folate nutritional status is important in minimizing developmental and degenerative disease. Therefore, constant monitoring of folate intake and of biomarkers of folate nutritional status is essential. The objective of this research was to compare two folate intake instruments and validate each one against RBC folate measured by a high-throughput liquid chromatography tandem mass spectrometry (HT LC-MS/MS) method described in the companion paper (Owens, J. E.; Holstege, D. M.; Clifford, A. J. *J. Agric. Food Chem.* **2007**, *55*, 3292−3297). A food frequency questionnaire (FFQ) and a folate-targeted semiquantitative Block dietary folate equivalents (DFE) screener were compared and individually validated against an HT LC-MS/MS method. RBC folate was 1178 ± 259 nmol/L (mean ± SD) in a population of 337 normal adult subjects. Folate intakes were 556 ± 265 μg/day by the FFQ and 524 ± 276 μg/day by the DFE screener. Folate intakes by the DFE screener were approximately 34 μg less than by the FFQ (paired *t* test, $p < 0.01$), but the intake instruments were highly correlated for total folate intake ($r = 0.608$, $p < 0.01$). Correlations between instruments and RBC folate were low ($r < 0.35$) but strong ($p < 0.01$). ROC curve analysis indicates that the measurement of RBC folate by the HT LC-MS/MS method is a better predictive tool than are intake instruments for the evaluation of marginal folate status.

KEYWORDS: RBC folate; LC-MS/MS; analytical method; folate intake; FFQ; DFE screener

INTRODUCTION

Folates are important cofactors for one-carbon metabolism and for DNA base synthesis. Humans are unable to synthesize folate and depend on an adequate and constant intake. A daily recommended intake of 400 μg of dietary folate equivalents (DFE) from a mix of natural food folates, folic acid food fortificants, and folic acid supplements is advised by the U.S. Food and Drug Administration (*1*). Since 1998, the U.S. FDA has required that most cereal and grain products in the United States be fortified with folic acid (*1*) to lower the incidence of neural tube defects (NTDs) (*2*, *3*). An adequate intake by women of child-bearing age is especially important to minimize NTD risk.

The mandate to increase folate intake was successful as evidenced by the decrease in NTD affected births (*4*) and reflected by the near doubling of red blood cell (RBC) folate levels post-fortification (*5*). This response in RBC folate to fortification was higher than predicted and may be due to higher than expected intakes of folic acid fortified foods or a significant excess fortification of such products (*5*, *6*). At the same time, some uncertainty persists concerning the actual increase in folate intake of Americans (*7*−*9*).

Dietary assessment instruments such as the food frequency questionnaire (FFQ), weighed food records, 24 h food recalls, and a newly developed Block dietary folate equivalents (DFE) screener have been utilized to assess folate intake (*10*−*13*). FFQs are widely used, convenient to administer, and not unduly burdensome to study volunteers, but they rely on memory, and some questions posed may be open to interpretation (*14*). The Block FFQ, developed by NutritionQuest, has a high reliability and a moderate to high validity (*11*). The Block DFE screener (also developed by NutritionQuest) is a one-page instrument whose procedure can be completed in 6−12 min, thus facilitating use in large surveys. It provides estimates of total, supplement, fortificant, and natural food folate intake. Block DFE screener results have been validated against RBC folate levels (*13*) but not against other intake instruments.

The RBC folate level is a good biomarker for folate status because of its correlation with liver, a major store (*15*), and it reflects long-term intake (>3 months) (*16*), whereas plasma and serum folate values are commonly accepted to reflect recent dietary intake. Intracellular accumulation of folate, pursuant to increased intake, may be crucial to minimizing NTD risk (*17*). Because of the uncertainty and assay dependence of RBC folate methods (*13*, *18*, *19*), an accurate and precise method to quantitate RBC folate is needed. The relation between folate intake and RBC folate is of interest to monitor folate intake and to identify sub-optimal folate status.

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Table 1. Comparison of Folate Intake As Measured by FFQ and Block DFE Screener, Which Is Reported both as μg/Day and as μg of DFE/Day[a]

	FFQ			DFE screener				
	diet (μg/day)	supplement (μg/day)	total (μg/day)	natural folates from foods (μg/day)	folic acid fortificant (μg/day)	supplement (μg/day)	total (μg/day)	total (μg of DFE/day)
all subjects	403 ± 194	153 ± 168	556 ± 265	132 ± 56	191 ± 90	194 ± 246	524 ± 276	788 ± 454
women	384 ± 159	156 ± 167	540 ± 243	132 ± 56	181 ± 77	213 ± 257	531 ± 285	804 ± 470
men	440 ± 244	147 ± 170	587 ± 301	132 ± 57	210 ± 108	160 ± 221	511 ± 258	758 ± 423

[a] Measurements are reported as mean ± standard deviation.

Validated and easily administered instruments for folate intake coupled with accurate analytical methods for RBC folate are essential to monitoring folate requirements for optimal health. In this work, RBC folate values determined by a newly developed HT LC-MS/MS method (*20*) were compared with folate intakes using the Block DFE screener (referred to throughout as DFE screener) and the Block 98.2 FFQ (referred to throughout as the FFQ).

METHODS AND MATERIALS

Subject Enrollment. Institutional approvals were previously described (*20*) except with the following detail. Subjects were recruited by mailed and posted advertisements in the California counties of Yolo, Solano, Sacramento, and San Joaquin during May 2004 to August 2005. Three hundred and seventy eligible men and women enrolled in the study. Participants enrolled in one single clinic visit at the Ragle Human Nutrition Research Center at the University of California, Davis. Prior to the visit, participants in the study received a packet via U.S. mail containing information about the study, consent forms, fasting instructions, and three questionnaires to assess folate intake. Each person completed a one-page folate-targeted food/supplements screener (the Block DFE screener) (*13*) as well as a standardized self-administered food frequency questionnaire (the Block 98.2 FFQ) (*11*, *21*) and a vitamin supplement questionnaire to capture the intake of all dietary and herbal supplements. At the time of the scheduled visit, participants were interviewed about general medical, personal, and family histories and demographic information. A $15 gift certificate to a local supermarket or department store was given to each person at the end of the visit.

Folate Analysis by HT LC-MS/MS. Whole blood samples were collected, processed, and analyzed as previously described (*20*).

Statistical Analysis. Statistical analyses were conducted using StatView statistical software (SAS Institute, Abacus Concepts, Inc., Berkeley, CA). The predictive value of both survey instruments was analyzed using receiver operator characteristic (ROC) curve analysis (*22*). ROC curves were calculated using a web-based calculator (*23*). Using a cutoff value of 200 μg/day (the EAR for non-pregnant, non-lactating adults (*24*)), the areas under the curve (AUC) were determined. An AUC value of 1 indicates perfect diagnosis of a test, and an AUC of 0.50 indicates that the test in use does not have much diagnostic utility. Sensitivity refers to the test's ability to identify true positive cases (individuals with low folate status who are identified as such), and specificity is the ability of the test to identify true negative cases (individuals with adequate folate status who are identified as such). AUC, sensitivity, and specificity values were reported for the survey instruments.

RESULTS AND DISCUSSION

Population Characteristics. Three hundred and seventy subjects enrolled in the study (240 women and 130 men). The mean age of the population was 44.3 years ± 12 (mean ± SD) with a range of 18−67 years. The racial/ethnic distribution of the population was 7.0% Hispanic/Latino, 8.4% Asian, 3.8% Black/African American, 71% Caucasian, 6.8% Mixed, 1.1% Native Hawaiian/Pacific Islander, and 1.6% Native American.

RBC Folate. Whole blood samples were analyzed for RBC folate concentration by a HT LC-MS/MS method for 337 subjects. The RBC folate concentrations for the remaining 33 subjects could not be reported due to missing hematocrit values. The mean RBC folate by HT LC-MS/MS was 1178 nmol/L ± 259 ranging from 524 to 2301 nmol/L.

Folate Intake. For the population of 370 volunteers, the mean folate intake was 556 ± 265 and 524 ± 276 μg/day (mean ± SD) by the FFQ and DFE screener, respectively (**Table 1**). The mean folate intake assessed by the FFQ for women was 540 ± 243 and 587 ± 301 μg/day for men (column 3, **Table 1**). The mean folate intake determined by the DFE screener was 531 ± 285 μg/day for women and 511 ± 258 μg/day for men (column 7, **Table 1**).

Unlike the FFQ, the DFE screener can also be used to differentiate between natural folate and the fortificant folic acid that contribute to dietary folate (columns 4 and 5, **Table 1**). The total percentage of fortificant folic acid in the diet and supplements was calculated. For this population of normal adults, 70.0 ± 14.3% (mean ± SD) of the ingested folate was folic acid. Only 30 ± 14.3% of the daily ingested folate was from natural folate.

While the DFE screener can report total folate intake in micrograms per day (column 7, **Table 1**), the total folate intake can also be reported as micrograms of DFE per day. The DFE screener considers differences in bioavailability between food folate (50% bioavailable) and folic acid (85% bioavailable) by using the factor 85:50 = 1.7 for folic acid (*24*). Therefore, total folate intake can be computed as micrograms of DFE per day = 1.0 × natural food folate intake (μg/day) + 1.7 × folic acid intake (μg/day), and thus, folate intake expressed as micrograms of DFE per day is greater than micrograms of folate per day. The folate form in food is mostly 5-methyltetrahydrofolate, whereas the folate form in fortificants and supplements is folic acid, a fully oxidized form. In this population, the mean folate intake using the DFE screener was 788 ± 454 μg of DFE/day for all participants, 804 ± 470 μg of DFE/day for women, and 758 ± 423 μg of DFE/day (mean ± SD) for men (column 8, **Table 1**).

Comparison of Folate Intake Instruments. The correlation of the FFQ versus the DFE screener for estimating intakes of total folate and dietary folate is shown in panels **A** and **B** of **Figure 1**. Results of both dietary instruments correlated well for total folate intake ($r = 0.608$, $p < 0.0001$), and this correlation compares well with prior reports (*10*, *11*, *25*−*27*). For folate derived from the diet, which includes natural folate and folic acid added as a fortificant, the dietary instruments also correlated well ($r = 0.533$, $p < 0.0001$).

Comparison of Folate Intake with RBC Folate Concentration. RBC folate reflects intracellular and tissue folate stores, and it is a better biomarker of folate nutritional status. The correlations of RBC folate versus total folate intake (in μg/day) by the DFE screener ($r = 0.335$, $p < 0.0001$) and versus the

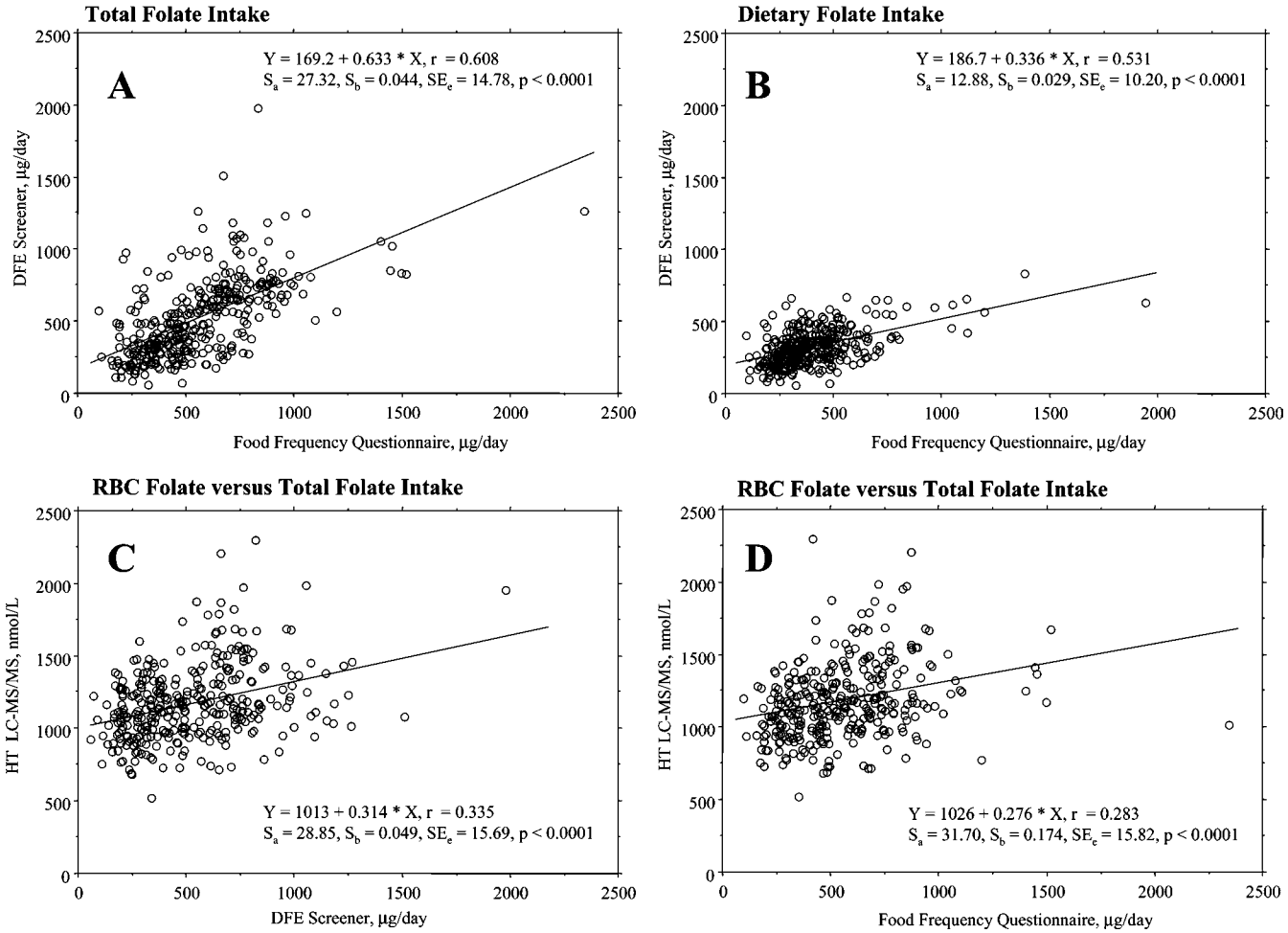

Figure 1. Correlation plots comparing the Block 98.2 food frequency questionnaire (FFQ) vs the recently developed semiquantitative folate-targeted Block DFE screener (DFE screener) for both total folate intake and dietary folate intake (which includes natural folates and the fortificant folic acid) to RBC folate as measured by HT LC-MS/MS for 370 normal volunteers. S_a, S_b, and S_e refer to the standard error of the intercept, slope, and estimate, respectively.

FFQ ($r = 0.283$, $p < 0.0001$) were both low yet strong (panels **C** and **D** of **Figure 1**). The correlation of RBC folate and total folate intake (expressed as micrograms of DFE/day) by the DFE screener was 0.338 ($p < 0.0001$) (correlation plot not shown). These correlations between folate intakes and RBC folate are consistent with previous reports (*10*, *12*, *13*, *27*−*29*). The highest reported correlation of RBC folate with folate intake (expressed as micrograms of DFE/day) was 0.55 in a cohort of pregnant Danish women (*30*). Serum or plasma folate reflect recent dietary intake of the vitamin, and in a recent study utilizing the NHANES 1988−1994 cohort, three dietary pattern indices had a mean correlation of 0.33 ± 0.05 with serum folate values (*31*). Thus, the correlation values between the HT LC-MS/MS method for RBC folate analysis and the FFQ and DFE screener compare well with previous reports.

ROC Curve Analysis. Using ROC curve analysis, the HT LC-MS/MS method for quantitation of RBC folate was utilized as a reference method for folate status and compared with folate intake as assessed by the FFQ and the DFE screener. The AUC for the FFQ was 0.678, and the sensitivity and specificity of this instrument were 12 and 96%, respectively. The DFE screener had an AUC of 0.661 but had the same sensitivity (12%) and lower specificity (91%) than the FFQ (*23*). Thus, both survey instruments are useful in screening subjects in this population with folate intakes above 200 µg/day (specificity >91%), but these instruments were poorer at positively identifying participants with intakes less than the EAR because of their low sensitivity (12%). The DFE screener takes less time to complete and has a greater potential use in screening the folate status of populations.

Conclusion. The present study demonstrates that the DFE screener produces estimates that are similar to that of an established FFQ. The DFE screener compares with RBC folate levels, a biomarker of folate nutritional status. Moreover, the DFE screener accounts for bioavailability differences between reduced natural folates and synthetic folic acid, indicating that the major source (70%) of ingested folate is folic acid in this population. The DFE screener may be especially useful when there is a need to focus on a single nutrient for repeat measurements to monitor changes in intakes over time. On the basis of the ROC curve analysis, the HT LC-MS/MS method is a better predictive tool of an individual's folate status than are intake instruments for the evaluation of marginal folate status. With further evaluation and updating of the food composition databases used to arrive at the intakes, these intake records may become highly useful and important tools to monitor folate status without a biomarker measurement.

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