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Validation of the folate food frequency questionnaire with serum and erythrocyte folate and plasma homocysteine

IRENA COLIĆ BARIĆ, ZVONIMIR ŠATALIĆ, IRENA KESER, IVANA CECIĆ & MARTINA SUČIĆ

Laboratory for Food Chemistry and Nutrition, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

Abstract

The aim of the present study was to develop and validate a folate food frequency questionnaire (FFQ)[1] designed to measure dietary folate equivalents. The self-administered FFQ containing 39 items and a reference period of 1 month (i.e. the previous month), was validated against three biomarkers: serum and erythrocyte (RBC) folate, and plasma homocysteine (Hcy). Subjects were women ($n = 99$) between the ages of 21 and 87 years. The Pearson correlation coefficients for folate intake and biomarkers were 0.36, 0.34 and –0.25 for serum and RBC folate, and plasma Hcy, respectively. A principal component factor analysis was performed on the three biomarkers to calculate the folate status factor. The Pearson correlation for the folate status factor and folate intake was 0.39. The FFQ described in this study is a valid tool for measuring folate intake expressed as dietary folate equivalents in adult women and is suitable for future investigations about the relationship between folate and disease, or as an educational tool.

Keywords: *Food frequency questionnaire, dietary folate equivalents, folate, biomarkers, homocysteine*

Introduction

Folate is the generic term for a water-soluble B complex vitamin that functions in single-carbon transfer reactions and exists in many chemical forms (Wagner 1996). The nutritional status of the vitamin is considered to be a potential public health issue requiring further study (Life Sciences Research Office 1989).

Adequate folate intake has an important role in the primary prevention of several diseases (Bailey and Gregory 2006), and adequate folate intake is especially important during the periconceptional period as a critical factor in the prevention of spina bifida and other neural tube defects (Botto et al. 1999).

Inadequate folate intake first leads to a decrease in serum folate concentration, then to a decrease in erythrocyte (RBC) folate concentration, followed by a rise in homocysteine (Hcy) concentration and megaloblastic changes in bone marrow and other tissues with rapidly dividing cells (Institute of Medicine 1999).

Correspondence: Irena Colić Barić, Laboratory for Food Chemistry and Nutrition, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia. E-mail: icolic@pbf.hr

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Carefully designed questionnaires can provide reasonably good, although not perfect, measures of dietary composition as assessed by comparison with more detailed assessments of diet (Willett 2001). The aim of this study was to develop and validate a self-administered folate food frequency questionnaire (FFQ), designed to measure dietary folate equivalents (DFE). The DFE express all forms of dietary folates, including synthetic folic acid used in fortified foods and dietary supplements, as an amount that is equivalent to naturally occurring food folate (Bailey 1998).

FFQs are both population and gender specific (Cade et al. 2002), so the FFQ was developed *de novo* instead of using an already existing form from another population validated folate FFQ (Pufulete et al. 2002; Yen et al. 2003; Owens et al. 2007; van de Rest et al. 2007; Verkleij-Hagoort et al. 2007). The target population was Croatian women.

Whenever a new questionnaire is designed, the authors are bound to consider its validation (Wise and Birrell 2002). In this study, the FFQ was validated against relevant biomarkers: serum and RBC folate and plasma Hcy. An advantage of using biomarkers is that they provide an objective measure of nutrient intake, with measurement errors essentially independent from those associated with dietary intake measures based on self report (van't Veer et al. 1993).

The RBC folate level is a good biomarker for folate status because of its correlation with the liver, a major folate store (Wu et al. 1975), and it reflects long-term intake (>3 months) (Jacob et al. 1998), whereas plasma and serum folate values are commonly accepted to reflect recent dietary intake. RBC folate is an indicator of long-term status because folate is absorbed only by the developing erythrocyte in the bone marrow and not by the circulating mature erythrocyte during its 120-day lifespan. The plasma concentration of Hcy (Krumdieck 1990) serves as an additional good indicator of folate nutritional status. Hcy increases when there is a deficiency of 5-methyl-tetrahydrofolate, necessary to convert Hcy to methionine. The inverse association between blood folate and plasma Hcy concentrations is well established (Institute of Medicine 1999).

FFQs are designed to rank individuals rather than to assess their absolute intake levels (Masson et al. 2003). The FFQ described in this study will be used in both epidemiological and clinical studies and as an educational tool for discriminating subjects with adequate and inadequate folate intake.

Subjects and methods

Subjects

The subjects ($n=99$) were women between the ages of 21 and 87 years (Table I) recruited from the university community (professors, staff and their families and

Table I. Descriptive characteristics of the study population and biomarkers ($n=99$).

Parameter	Mean	Standard deviation
Age (years)	52.3	15.97
Body mass index (kg/m^2)	25.3	4.52
Current smokers (% subjects)	31.3	
Folate intake (μg DFE/day)	252.6	184.79
Serum folate (nmol/l)	21.5	7.27
Erythrocyte folate (nmol/l)	1,065.5	338.92
Plasma homocysteine (μmol/l)	10.0	2.73

friends). Exclusion criteria included special diets (vegetarian and weight-loss diets), use of drugs known to interfere with folate metabolism (Institute of Medicine 1999), and diseases known to alter folate metabolism including rheumatoid arthritis and dermatological conditions like psoriasis (Gisondi et al. 2007; Woolf and Manore 2008). Vegetarians are at risk for increased Hcy levels because of inadequate B_{12} intake, and weight reduction can adversely affect Hcy metabolism (Henning et al. 1997; Stabler and Allen 2004).

Participation was voluntary and all participants signed an informed consent form. The Ethical Committee of the Institute for Medical Research and Occupational Health approved the study protocol.

Food frequency questionnaire

The FFQ is a 39-item questionnaire that uses the previous month as a reference period with the following consumption frequencies: never, 1/month, 2–3/month, 1/week, 2–3/week, 4–6/week and every day. The 39 separate food items were dairy products (four items), eggs (one item), meat and fish (seven items), legumes (three items), grain products (three items), vegetables (nine items), fruit (seven items), cacao products (two items), vitamin drink (the brand most commonly consumed in Croatia) and breakfast cereals (corn flakes and muesli). The food items list was based on Croatian food composition tables (Kaić-Rak and Antonić 1990).

The subjects received the FFQ in the form of a booklet with incorporated food photographs (Senta et al. 2004). Each photograph showed small, medium, and large portion sizes. The use of a set of photographs is associated with smaller errors in portion size perception than when using individual photographs or no photographs at all (Nelson et al. 1994). To avoid overestimation and underestimation of portion size, subjects were also asked to describe their usual intake alternatively by stating intake in grams or size descriptions using kitchen utensils.

Prior to administration, the FFQ was tested in a pilot study in which subjects ($n = 25$) completed a draft version of it and were interviewed on aspects of comprehension, helpfulness of portion estimation aids, and adequacy of consumption frequencies. Amendments were minor and included more precise descriptions of portion sizes for some food items.

Folate intake was calculated using national food composition tables (Kaić-Rak and Antonić 1990). Folate is heat labile (Herbert 1985) so retention factors were taken into account (Kaić-Rak and Antonić 1990). Beside food folate, folic acid originating from fortified foods and dietary supplements was also assessed. At the moment, folate enrichment of for example cereals is not mandatory and not practiced by Croatian food producers.

The subjects provided detailed information on supplement use and consumption of folate-fortified food products (breakfast cereals, milk and vitamin drinks). Self-reported dietary supplement use (including folate) is considered valid and has been confirmed by biological markers (Brantsaeter et al. 2007).

The FFQ was designed to measure DFE. The use of DFE is recommended for evaluating the adequacy of folate intake (Suitor and Bailey 2000). DFE are units that account for the differences in the absorption of food folate and of synthetic folic acid obtained from dietary supplements of foods fortified with folic acid. Folate found in fortified foods or supplements is estimated to be 1.7 times more bioavailable than

naturally occurring folate, and folate in a supplement form taken on an empty stomach is two times more bioavailable. Thus, to calculate the DFE, the folate content of foods fortified with folate and the folate content of dietary supplements were multiplied by 1.7 and the folate content of dietary supplements taken on an empty stomach was multiplied by two.

Biomarkers

Overnight fasting blood samples were drawn in order to measure serum and erythrocyte (RBC) folate and plasma Hcy. Folate and Hcy were determined using Abbott AxSYM systems (Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA), according to the manufacturer's instructions (Shipchandler and Moore 1995; Manzella et al. 1999). Cut-off values for the folate level (16 nmol/l and 572 nmol/l for serum and RBC folate, respectively) were defined by the manufacturer of the commercial kit and the cut-off value for plasma Hcy was defined as 15 μmol/l (Refsum et al. 2004).

Statistical analyses

Several methods were used to assess the validity of the FFQ. A principal component factor analysis was performed on three biomarkers. Variance of each variable in a certain set of variables (in our study set of folate biomarkers) can be divided on common variance (variance shared with other variables within the data-set) and unique variance (sum of specific variance and error variance). The first principal component was calculated in order to represent as much as possible common variance of a certain data-set. We assume that common variance of folate biomarkers represented by the first principal component is the best way to express overall folate status. Pearson product-moment correlation coefficients were calculated for folate intake based on the FFQs and respective biomarkers of folate status. Sensitivity (the ability of the FFQ to identify individuals with low folate status) and specificity (the ability of the FFQ to identify individuals with adequate folate status) were calculated using a 200 μg/day cut-off value for folate intake, 15 μmol/l for plasma homocysteine, 16 nm/l for serum folate and 572 nmol/l for RBC folate. The ability of the FFQ to correctly classify participants into quartiles of every respective biochemical indicator was also determined. Percentages of those correctly classified (participants classified within the same quartiles), closely classified (participants classified within the same or adjacent quartiles) and grossly misclassified (participants classified in the opposite quartiles) and corresponding quadratic weighted kappa values were calculated.

STATISTICA 7.1 was used for the statistical analysis (StatSoft, Inc. 2005). The Kolmogorov–Smirnov test was used for testing the normality of data distribution. A P value less than 0.05 was considered statistically significant.

Results

Plasma Hcy, serum, and RBC folate levels were normally distributed. The distribution of folate intakes estimated by the FFQ was right-skewed and was therefore log-transformed. The median of folate intake was 204 μg DFE.

High correlation coefficients for all biomarkers with the first principal component imply that all three biomarkers truly measure the same parameter (Table II). A high percentage of the total variance explained by the first principal component confirms this, so the factor scores could be considered a measure of 'true' folate status.

Pearson product-moment correlation coefficients for biomarkers and folate intake were statistically significant (Table III). Biomarkers for long-term (RBC folate) and short-term folate intake (serum folate) did not differ significantly.

The sensitivity and specificity of the folate FFQ for the cut-off value of 200 μg DFE are presented in Table IV. The 200 μg folate cut-off value was set as the recommended folate intake for adults in Croatia (Ministarstvo zdravstva i socijalne skrbi 2004). The quadratic weighted kappa for all biomarkers and folate status factor was above 0.2 and the gross misclassification of subjects into quartiles was less than 10% (Table V). In total, 5.1% of subjects reported consumption of folic acid fortified milk that recently appeared on the Croatian market, and 7.1% of subjects reported use of supplements containing folic acid.

When supplement users and consumers of folic acid fortified foods (breakfast cereals, milk and vitamin drinks) were excluded, correlations between folate intake and biomarkers were as follows: 0.20, 0.28, –0.23 and –0.30 for serum and RBC folate, Hcy and folate status factor, respectively. The correlations were not statistically significant ($P > 0.05$), probably because of the relatively small number of subjects ($n = 35$), so these results should not be generalized.

Discussion

The aim of this study was to report the validity of a *de novo* FFQ designed for measuring folate intake in adult Croatian women. The target population was women only as folate FFQs have been shown to be strongly gender specific (Pufulete et al. 2002; van de Rest et al. 2007).

The FFQ was validated against relevant biomarkers of folate status: serum and RBC folate and plasma Hcy. Serum folate indicates recent dietary folate intake, and RBC folate is an indicator of long-term status. In this study, the correlation for folate intake based on the FFQ and serum and RBC folate did not differ significantly, although the correlation was somewhat higher for serum folate. This can be explained through the defined reference period of the FFQ, which was the previous month. Although it is not the most recent period, it is shorter than 3 months—which is a mature erythrocyte's lifespan.

In addition to blood folate concentrations, it is important to also evaluate folate status indices that may indicate changes in metabolic function, such as Hcy. Use of Hcy to identify preclinical folate deficiency has been recommended on the grounds

Table II. Principal components factor analysis of folate status biomarkers ($n = 99$).

Factor structure matrix	Folate status factor
Serum folate	0.81
Erythrocyte folate	0.84
Plasma homocysteine	−0.77
Eigenvalue	1.96
Total variance explained (%)	65

Table III. Pearson product-moment correlation coefficients ($n=99$).

Parameter	Folate intake ($\log_{10}$[DFE (μg/day)])
Serum folate	0.36
Erythrocyte folate	0.34
Plasma homocysteine	−0.25
Folate status factor	0.39

$P<0.05$ for all correlation coefficients.

that Hcy is a more sensitive marker than B vitamin levels (Refsum et al. 2004). The correlation of folate intake and Hcy level was negative as expected, but was lower than the correlation with blood folate. Use of three biomarkers enabled the calculation of a folate status factor that had higher correlation with folate intake than any of the biomarkers used ($r=0.39$; $P<0.0001$).

Dietary folate intake measured by the FFQ was expressed as DFE, which takes into account data indicating that synthetic folate is better absorbed than naturally occurring folate (Sauberlich et al. 1987; Pfeiffer et al. 1997). When synthetic folic acid is consumed as a supplement without food, it is nearly 100% bioavailable (Gregory 1997). A recent long-term feeding study supports a 1.7 equivalency factor used to calculate DFE (Yang et al. 2005).

An important issue regarding the estimation of folate intake is the quality of data from food composition tables. Traditional methods for food folate analysis have limitations and probably underestimate the true folate content. The food composition tables used in this study are outdated (Kaić-Rak and Antonić 1990) so new food folate values based on direct analysis with validated assay procedures are both greatly needed and expected. However, intakes derived from the food frequency method proved effective in predicting folate status (Jacques et al. 1993). Thus, food composition data are suitable for accurately classifying folate intakes, although quantitative estimates might be underestimated (Bailey and Gregory 2006).

One limitation of the study is that genetic factors were not taken into account. Genetic factors have been shown to affect the responsiveness of serum folate to diet alterations and supplement use (Silaste et al. 2001; Fohr et al. 2002). A recent Croatian study reported that less than 10% of individuals are homozygous regarding 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphism (Lovricevic et al. 2004). The MTHFR polymorphism has been associated with significantly increased plasma Hcy and altered folate metabolism (Bailey and Gregory 2006).

When compared with recently published validation studies of folate FFQs, our results compare well. One of the highest reported correlations of RBC folate with folate intake (DFE) was 0.55 among pregnant Danish women (Mikkelsen et al. 2006). The folate FFQ developed for the Dutch elderly showed a weak correlation between folate intake and biomarkers ($r=0.14$, 0.05 and 0.02 for serum and RBC folate and

Table IV. Sensitivity and specificity (%) of the folate FFQ for a cut-off value of 200 μg DFE ($n=99$).

Parameter	Cut-off value	Sensitivity (%)	Specificity (%)
Serum folate	16 nmol/l	61	56
Erythrocyte folate	572 nmol/l	70	54
Plasma homocysteine	15 μmol/l	100	54

Table V. Cross-classification (%) into quartiles for folate intake and biomarkers and weighted kappa ($n = 99$).

Folate intake	Same quartile	Same or adjacent quartile	Opposite quartile	Quadratic weighted kappa
Serum folate	33	70	6	0.24
Erythrocyte folate	32	68	3	0.28
Plasma homocysteine	32	67	6	0.20
Folate status factor	31	72	5	0.28

Hcy, respectively) but the FFQ was able to correctly classify subjects according to their folate intake (van de Rest et al. 2007). Owens et al. (2007) reported a correlation for RBC folate and folate intake (DFE) under 0.35 for the two questionnaires tested (DFE screener and FFQ). The DFE screener sensitivity was 12% with a specificity of 91%. The FFQ sensitivity was 12% with a specificity of 96%. Among Dutch women of reproductive age, the FFQ was validated against serum and RBC folate (Verkleij-Hagoort et al. 2007). Pearson correlations were 0.20 (FFQ versus serum folate) and 0.28 (FFQ versus RBC folate). Among adolescent females aged 16–19 the FFQ was validated against serum and RBC folate (Green et al. 1998). For serum folate, the FFQ classified 45% of subjects correctly into quartiles, 80% were correctly and closely classified and 20% were misclassified (not within the same or adjacent quartile). For RBC folate, the FFQ classified 32% of subjects correctly and 75% correctly and closely, while 25% of subjects were misclassified. Among women between the ages of 21 and 47 years, the correlation for folate FFQ and Hcy was –0.26 (Yen et al. 2003).

If the possibility of false-negative associations between diet and disease in epidemiological studies is to be minimized, weighted kappa values of above 0.4 are desirable for nutrients of interest (Masson et al. 2003). Values of kappa over 0.80 indicate very good agreement, values between 0.61 and 0.80 show good agreement, from 0.41 to 0.60 show moderate agreement, from 0.21 to 0.40 show fair agreement, and <0.20 show poor agreement (Altman 1991). However, these criteria apply for relative validity (i.e. for comparison of two dietary assessment methods). In this study, the FFQ was validated against biomarkers and the weighted kappa ranged from 0.20 to 0.28 but it would be reasonable to expect higher kappa values if comparison was made with dietary records, for example. When two dietary assessment methods are compared they have similar biases, which could lead to overestimation of the agreement between the reference method and the FFQ (Ocké and Kaaks 1997; Wise and Birrell 2002). When biomarkers are used, errors in the determination are not correlated with errors in the data obtained by the FFQ so the resulting agreement is likely to be weaker.

Based on the results of this validation study, it can be concluded that the developed folate FFQ is a valid tool for measuring DFE intake in adult Croatian women. A recent Croatian study showed inadequate folate intakes in women between the ages of 20 and 30 years, and one of the applications of the FFQ might be in dietary counselling or public health programmes with the aim of improving folate status (Pucarin-Cvetkovic et al. 2006).

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Note

1 The FFQ may be obtained from the authors upon request.

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