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Validation of the folate food frequency questionnaire in vegetarians

IRENA COLIĆ BARIĆ[1], ZVONIMIR ŠATALIĆ[1], ŽELJKO PEDIŠIĆ[2], VESNA ŽIZIĆ[3] & IRENA LINARIĆ[3]

[1]*Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia,* [2]*Faculty of Kinesiology, University of Zagreb, Zagreb, Croatia, and* [3]*Children's Hospital Zagreb, Zagreb, Croatia*

Abstract

Adequate folate status has an important role in the prevention of chronic and developmental diseases and is considered a potential public health issue. Therefore, valid tools for measuring the vitamin intake are needed. In our previous study a folate food frequency questionnaire[1] (FFQ) designed to measure dietary folate equivalents was developed and validated among adult women against serum and erythrocyte (red blood cell) folate and plasma homocysteine. The aim of the present study was to validate the FFQ in vegetarians ($n = 75$). The Pearson correlation for folate intake and biomarkers was 0.41, 0.36 and -0.15 for serum and red blood cell folate and plasma homocysteine, respectively. The quadratic weighted kappa value for biomarkers was above 0.2 and the gross misclassification of subjects into quartiles was less than 10%. The FFQ is a valid tool for measuring dietary folate equivalent intake in Croatian vegetarians.

Keywords: *Food frequency questionnaire, validation, folate, vegetarians*

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 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 (red blood cell [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, Food and Nutrition Board 1999).

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In our previous report (Colić Barić et al. in press), development of a folate-specific self-administered food frequency questionnaire (FFQ) was described and validated in

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

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a population of adult women. The FFQ measures dietary folate equivalents (DFE) that 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).

The aim of the present study was to assess the validity of the FFQ in a population with specific dietary patterns; namely, vegetarians. Vegetarians are expected to have superior folate status to omnivores due to higher vegetable consumption (Majchrzak et al. 2006). Folate is heat labile and has a short shelf-life, and fresh fruit and vegetables are excellent dietary sources (Kaić-Rak and Antonić 1990). Other important sources in a vegetarian diet include legumes and nuts.

Subjects and methods

Subjects

The subjects ($n = 75$) were vegetarians of both genders (13 males and 63 females) and varying dietary patterns (21 vegans and 54 laco-ovo-vegetarians) (Table I). Veganism was defined as avoidance of any foods of animal origin, whereas lacto-ovo vegetarians consumed milk, eggs and dairy products (Position of the American Dietetic Association and Dietitians of Canada 2003).

Subjects were recruited through local vegetarian societies. Exclusion criteria included use of drugs known to interfere with folate metabolism and following a vegetarian diet for less than 12 months (Institute of Medicine, Food and Nutrition Board 1999). 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

AQ1 The details of the FFQ were published previously (Colić Barić et al. in press). The FFQ is a 39-item questionnaire with the previous month as a reference period and the following available consumption frequencies: never, one/month, two to three/month, one/week, two to three/week, four to six/week and every day. The 39 separate food

Table I. Descriptive characteristics of the study population and biomarkers ($n = 75$).

Parameter	Mean	Standard deviation	Normal value
Age (years)	35.4	9.15	
Body mass index (kg/m^2)	22.0	3.09	
Females (% subjects)	82.7		
Males (% subjects)	17.3		
Vegans (% subjects)	28.0		
Lacto-ovo-vegetarians (% subjects)	72.0		
Current smokers (% subjects)	14.7		
Folate intake (μg DFE/day)	311.8	191.35	200[a]
Serum folate (nmol/l)	28.8	6.19	16–35[b]
Erythrocyte folate (nmol/l)	922.8	290.69	572–1843[b]
Plasma Hcy (μmol/l)	9.8	2.75	5–15[b]

[a]Recommended folate intake for adults in Croatia (Ministarstvo zdravstva i socijalne skrbi 2004). [b]Defined by the manufacturer of the commercial kit (Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA).

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 (one brand most commonly consumed in Croatia) and breakfast cereals (cornflakes and muesli). The subjects received the FFQ in the form of a booklet with incorporated food photographs (Senta et al. 2004). Folate intake was calculated using national food composition tables taking into account retention factors since folate is heat labile (Kaić-Rak and Antonić 1990; Agte et al. 2002). 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.

Subjects provided detailed information on supplement use and consumption of folate-fortified food products currently available at the Croatian market; namely, breakfast cereals, milk (two brands) 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 measures DFE. The use of DFE is recommended for evaluating the adequacy of folate intake (Suitor and Bailey 2000). 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 2.

Biomarkers

Blood samples were drawn following an overnight fast in order to measure serum and RBC folate and plasma Hcy. Folate and Hcy were determined using Abott 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 and 572 nmol/l for serum and RBC folate, respectively) were defined by the manufacturer of the commercial kit and the cut-off for plasma Hcy was defined as 15 μmol/l (Refsum et al. 2004). The values for serum and RBC folate represent a minimal normal value, and the value for plasma Hcy represents a maximal normal value.

Statistical analyses

Several methods were used to assess the validity of the FFQ. A principal component factor analysis was performed on three biomarkers. The 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 (the 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 the 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 FFQ's 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 Hcy, 16 nm/l for serum folate and 572 nmol/l for RBC folate. The ability of the FFQ to correctly

Table II. Principal components factor analysis of folate status biomarkers ($n = 75$).

Factor structure matrix	Folate status factor
Serum folate	0.85
Erythrocyte folate	0.85
Plasma Hcy	−0.64
Eigenvalue	1.83
Total variance explained (%)	61

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 the corresponding quadratic weighted kappa values were calculated.

STATISTICA 7.1 was used for the statistical analysis (StatSoft, Inc. 2006). The Kolmogorov–Smirnov test was used for testing the normality of data distribution. $P < 0.05$ 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 first principal component was highly correlated with all biomarkers (Table II). The Pearson product-moment correlation coefficients for biomarkers and folate intake were statistically significant for serum and RBC folate, but not for plasma Hcy (Table III).

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).

Discussion

Few FFQs have been created or modified to measure dietary intake among vegetarians (Waldmann et al. 2006) and, to our knowledge, this is the first study reporting the validity of a folate FFQ in vegetarians. The FFQ was already validated among adult women, but the FFQ is population specific and it is important to check its validity in

Table III. Pearson product-moment correlation coefficients with 95% confidence interval ($n = 75$).

Parameter	Folate intake ($\log_{10}$[DFE (μg/day)])
Serum folate	0.41* (0.20–0.58)
Erythrocyte folate	0.36* (0.15–0.54)
Plasma Hcy	−0.15 (−0.36–0.08)
Folate status factor	0.41* (0.20–0.58)

*$P < 0.05$.

Table IV. Sensitivity and specificity (%) of the folate FFQ for cut-off value 200 μg DFE and absolute frequencies for each category ($n=75$).

		Folate intake			
Parameter	Cut-off value	<200 μg DFE	>200 μg DFE	Sensitivity	Specificity
Serum folate	<16 nmol/l	0	1	0	69
	>16 nmol/l	23	51		
Erythrocyte folate	<572 nmol/l	3	5	38	70
	>572 nmol/l	20	47		
Plasma Hcy	>15 μmol/l	0	4	0	68
	<15 μmol/l	23	48		

vegetarians, who are usually excluded from the FFQ validation studies because of having a specific diet (Cade et al. 2002; Wise and Birrell 2002).

The use of biomarkers to validate dietary intake methods is increasing (Johnson et al. 2008). Validation was performed through comparison of folate intake and 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). Without the existence of the relevant biomarkers, the alternative would be relative validity; that is, comparison of the FFQ with other dietary assessment methods, which is the case with nutrients such as calcium (Subar 2004; Šatalić et al. 2007).

The RBC folate level is a good biomarker for folate status because of its correlation with the liver, a major folate storage site (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 RBC in the bone marrow and not by the circulating mature RBC 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.

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 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 RBC's lifespan.

Table V. Cross-classification (%) into quartiles for folate intake and biomarkers and weighted kappa values ($n=75$).

Folate intake	Same quartile	Same or adjacent quartile	Opposite quartile	Quadratic weighted kappa
Serum folate	36	77	4	0.39
Erythrocyte folate	33	77	4	0.37
Plasma Hcy	28	75	8	0.24
Folate status factor	41	83	7	0.42

The correlation of folate intake and Hcy level was negative as expected, but lower than the correlation with serum and RBC folate, and not significant. When the FFQ was administered for validation purposes among adult women (Colić Barić et al. in press), the negative correlation between folate intake and plasma Hcy was significant. The missing correlation in vegetarians can be explained by the high probability of their inadequate B_{12} status, which can elevate their Hcy in spite of adequate folate intake (Stabler and Allen 2004). Also, it is generally agreed that plasma Hcy is most useful as a functional measure of folate status when the concentration of folate in the diet is in the low to moderate range, which is not the case in the vegetarian diet (O'Leary and Sheehy 2002; Li et al. 2000).

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). A 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 Hcy) 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 years, the FFQ was validated against serum and RBC folate (Green et al. 1998). For serum folate, the FFQ classified 45% of the 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).

Pearson's correlation, weighted kappa and classification into quartiles were more favourable in vegetarians than among omnivore women in the previous validation study (Colić Barić et al. in press). This is not surprising since vegetarians are more health conscious and therefore more focused on personal diet, and it was shown that vegetarians are able to recall their diet with higher reliability (Willett 1999; Kuzma and Lindsted 1990).

Based on the results of this validation study, it can be concluded that the folate FFQ is a valid tool for measuring DFE intake in adult Croatian vegetarians.

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Note

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

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