DOI 10.1002/mnfr.201200105 *Mol. Nutr. Food Res.* 2013, *57*, 562–581

REVIEW

Dietary intake and biological measurement of folate: A qualitative review of validation studies

Jin Young Park[1], Stein Emil Vollset[2], Alida Melse-Boonstra[3], Véronique Chajès[4], Per Magne Ueland[5] and Nadia Slimani[1]

[1] Dietary Exposure Assessment Group, Nutrition and Metabolism Section, International Agency for Research on Cancer, Lyon, France
[2] Norwegian Institute of Public Health and University of Bergen, Kalfarveien, Bergen, Norway
[3] Division of Human Nutrition, Wageningen University, EV Wageningen, The Netherlands
[4] Biomarkers Group, Nutrition and Metabolism Section, International Agency for Research on Cancer, Lyon, France
[5] Section for Pharmacology, University of Bergen, New Lab Building, Bergen, Hordaland, Norway

Received: February 14, 2012
Revised: June 8, 2012
Accepted: August 1, 2012

Folate is a nutrient of major health significance, but its dietary intake assessment is particularly complex to quantify through traditional approaches. Attempts have been made to validate dietary instruments for assessing folate intake against circulating concentration biomarkers. However, this requires careful attention on various methodological issues. We conducted a qualitative review of 17 recently published validation studies to identify these issues. The majority of the tested instruments were self-administered food frequency questionnaires while the biomarker most frequently used was serum/plasma folate. Seasonality was not considered in most studies. Little attention was given to using updated food composition databases based on reliable chemical methods and including fortified foods and dietary supplements. Time sequence of the test instrument and the reference biomarker used was often ambiguous, and reference periods did not always match. Correlation coefficient was the metric most commonly used, and correlations between dietary folate intake and blood folate concentration varied from weak to moderate ($r = 0.05$–0.54). The correlations were stronger when dietary supplement use was considered, and when serum/plasma rather than red blood cell folate was used. This review summarises issues that need to be considered in future studies intending to validate instruments for dietary folate assessment against concentration biomarkers.

Keywords:
Biomarker / Dietary assessment / Folate / Validation / Validation studies

Additional supporting information may be found in the online version of this article at the publisher's web-site

1 Introduction

Folate, a water-soluble B vitamin, plays an important role in one-carbon metabolism [1]. Inadequate folate intake has been linked to the risk of anaemia [2], neuropsychiatric disorders [3] and neural tube defects [4]. It has also been shown that inadequate dietary intake of folate is associated with elevated plasma homocysteine concentrations, a factor that is associated with cardiovascular disease [5–7]. Furthermore, folate deficiency leads to misincorporation of uracil instead of thymine into human DNA and to an increased frequency of chromosomal breaks, causing disruption of DNA synthesis, repair and methylation [8], which may increase the risk to develop some cancers, in particular colorectal cancer [9]. Important food sources of folate include vegetables, especially green leafy vegetables, cereals, fruits, nuts and seeds and liver and its derived products [10,11]. In comparison with naturally occurring food folate, folic acid refers to the synthetic folate; it is chemically stable and rarely found in natural food, yet widely used for the purpose of food fortification and dietary supplements [12,13].

Multiple instruments have been used to assess dietary folate intake, with the food frequency questionnaire

Correspondence: Dr. Jin Young Park, Section of Nutrition and Metabolism, Dietary Exposure Assessment Group, International Agency for Research on Cancer, 150 cours Albert-Thomas, 69372 Lyon Cedex 08, France
E-mail: ParkJY@fellows.iarc.fr
Fax: +33-4-72-73-83-61

Abbreviations: 24HDR, 24-h dietary recall; **DFE**, Dietary Folate Equivalents; **FCM**, Food Choice Map; **FFQ**, food frequency questionnaire; **FIT**, Folate Intake Tool; **RBC**, red blood cell

(FFQ) being the most commonly used method in large epidemiological studies due to its cost-effectiveness in use and convenience in administration [14]. Another dietary instrument that is commonly used is the 24-h dietary recall (24HDR) that may provide more accurate estimates of intake for a specific recalled day [15]. Dietary folate intake has also been estimated using other instruments, such as dietary records (food diaries), weighed food records and dietary history methods [16]. Given the complexities in capturing dietary exposures, however, all these dietary assessment methods are associated with measurement errors [17]. In the absence of a good standard method that provides a true measure of dietary intake, the relative validity of a test dietary method has long been assessed by using a more intensive but presumably more accurate dietary reporting method [18, 19]. To be a valid reference instrument, errors in the reference method should be independent of those in the test method and also with the true intake [19, 20]. However, it is unlikely that these requirements are entirely fulfilled for the available reference dietary methods [19].

For this reason, biochemical markers (biomarkers) have been increasingly used in validation studies as a surrogate for actual dietary intake. These biomarkers may reflect recent or longer term intake and the bioavailability of the actual nutrient. Besides, their measurement errors are independent of those associated with self-reported dietary intake [17, 21].

The majority of biomarkers of diet/nutrient intake identified so far are based on the concentration of a specific substance in biological fluids or tissues [20]. For the case of folate, most studies have used the concentration of folate in blood (i.e., serum/plasma or red blood cell folate) as a biomarker of dietary folate, with the assumption that they are responsive to dietary intake in a dose-dependent manner [22]. Unlike recovery biomarkers such as doubly labeled water, urinary nitrogen/potassium, which provide an estimate of absolute quantitative intake levels of certain nutrients [21], the use of these concentration biomarkers does not allow direct validation of the dietary intake measured by other dietary instruments, but only provides a correlate of dietary intake level [23], as the quantitative relationship between these markers and dietary intake level is influenced by a number of physiological and environmental factors [20, 21, 24].

Nevertheless, there have been a few studies that 'validated' a dietary folate intake assessment method (predominantly FFQ) against these concentration biomarkers of folate, such as folate levels in serum/plasma or erythrocytes. Conventionally, these studies have relied on correlations between measures obtained by test and reference instruments and reported them as evidence of validity [19, 25]. However, not many studies paid careful attention to methodological and other critical issues like study design, use of food composition database, choice of the particular biomarker as a reference for the dietary intake, consideration of seasonal variation, analytical and laboratory issues and appropriateness of the statistical methods used. In this paper, we critically appraised recent studies that compared folate intake assessed by FFQ, 24HDR or other dietary instruments with folate concentration biomarkers, with the ultimate aims to provide suggestions for future studies that intend to compare dietary folate intake against concentration biomarkers to improve their design and facilitate the interpretation of their main outcomes.

2 Materials and methods

A search in the MEDLINE (http://www.ncbi.nlm.nih.gov/pubmed) and the Web of Science[SM] (http://apps.isiknowledge.com) databases was conducted up to September 2011 by using a combination of MeSH terms: Diet; 'Nutritional status'; 'Nutrition Assessment'; 'Nutritive Value'; 'Validation studies as topic'; 'Reproducibility of Results'; 'Folic acid'; 'Vitamin B complex'; 'Biological markers' and related key words in titles or abstracts: 'dietary intake'; folate; 'folic acid'; 'folic acid'; (substance); validation; validity; 'validation studies' (publication type); biomarkers and 'biochemical markers'. Reference lists of relevant articles were checked to identify any additional studies from the Web of Science[SM] using the general search, related records search and cited reference search functions. Relevant articles were included in this review if they reported on 'validation studies' of FFQ, 24HDR, food records or other forms of approach assessing dietary folate intake with folate-related biomarkers as a reference method.

We included articles written in English published since 2000 to conduct an in-depth review of more recent articles, which may reflect more recent analytical development, hence an improved precision in both dietary and biochemical assessment of folate. Studies of diseases and folate status, studies dealing with relative validation, studies in diseased, institutionalized persons, or pregnant women and reports only on statistical methodology were excluded. Figure 1 illustrates our search strategies, and selection and exclusion criteria.

Information extracted from each study included the first author, year, country where the study was conducted, characteristics of study participants, FFQ or test method validated, reference dietary method used (if applicable), food composition database, reference biomarker used, consideration of dietary supplement use in the study, main statistical method applied and main results on dietary folate assessments compared against reference dietary methods and biomarkers. Throughout the paper, a test method (often FFQ) is denoted as Q, while a reference dietary method is denoted as R and a biomarker as M. If a study used two biomarkers, they are denoted as M1 and M2, respectively. For convenience, the term 'red blood cell (RBC) folate' is used for erythrocyte folate.

3 Results

The initial search retrieved 363 articles. We identified a total of 29 relevant articles through cited reference search and hand search. After applying the exclusion criteria, 17 articles [26–42] published in the past 10 years were included (Fig. 1).

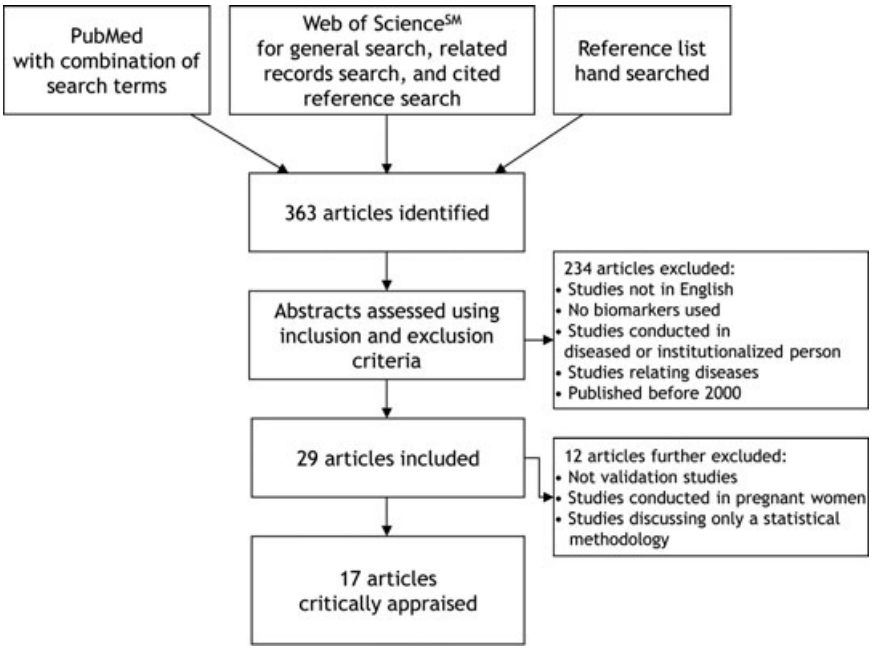

Figure 1. Flow chart of study selection and exclusion for the review of validation studies of dietary instruments for assessing folate intake against circulating concentration biomarkers.

We summarised characteristics of included studies in Table 1 and discussed each study more in detail in terms of choice of reference method, study administration, statistical analysis, main findings and limitations in Table 2.

3.1 Study participants

Sample sizes ranged from 28 [42] to 1281 [40]. The age of participants ranged between 18 and 87 years with four studies being conducted in women of childbearing age (18–35 years) [26, 30, 38, 41] and one study being conducted in elderly participants (50–75 years) [40]. Six studies were carried out in women only [26, 27, 30, 41–43] while two studies included men only [32, 33]. The majority of the studies were conducted in North America and Europe while few studies were conducted in Asia [32, 37] and Latin America [26]. While most of the studies explored validation of dietary instruments for folate intake only, few studies [30, 32, 34, 38, 39, 41] investigated folate together with other B vitamins or nutrients.

3.2 Test methods

3.2.1 Food items

Most of the validation studies used a FFQ as a test method, as summarised in Table 1. Exceptions to this were three studies that investigated the validity of newly developed instruments, namely Food Choice Map (FCM) [38], Folate Intake Tool (FIT) [31] and a focused recall [42]. While some studies tested existing general FFQs including more than 100 food items to assess folate intake, others tested simplified versions of FFQ [27, 28, 36, 40] or folate-focused instruments [31, 42], which were specifically developed to measure folate intake only. These folate-focused instruments had less number of items, ranging from 19 to 90, and it was not always clear how the food items were selected and to what extent those selected items covered the average dietary folate intake in the study population [27, 28, 42].

3.2.2 Frequency and portion size estimation

In most cases, test methods had the questions on pre-defined frequency and standard food portion size, while for the folate-specific recall approach, participants answered to open questions [42]. Although some of the food sources of folate are seasonally consumed, none of the test instruments included in the current review had a separate section addressing seasonality. Only a study by Shai et al. [37] collected three FFQs over 13 months and adjusted for seasonality by considering availability during the year (Table 2). Portion size estimation in the test instruments was often aided by either photo books or food models [26–28, 33, 36, 38].

3.2.3 Mode of administration

The majority of test instruments were self-administered (76%) while some instruments were based on either telephone/e-mail [42] or face-to-face interviews [33, 38, 39]. Apart from the study by van de Rest and colleagues [40], no studies that used self-administered test instruments reported on controlling for completeness and consistency. The FCM administered on an in-person interview required 50 min to

Table 1. Description of the 17 validation studies regarding folate intake included in the review

Study	Study participants Number (age range)	FFQ/test method		Reference dietary method		Food composition database	Reference biomarker method		Supplement use considered	Main statistical methods	Main results
		Number of items	Reference period	Method	Duration		Biomarkers used	Biochemical assays used			
Fayet et al. (2011), Australia	256 women (18–35 years)	235 (electronic)	Past 3 months	2 or 3 × 24HDRs by face-to-face interview ($n = 53$)	Within 2 weeks (1 to be a weekend day)	NUTTAB database of Australian food composition (2006)	Serum folate (M1, nmol/l) RBC folate (M2, nmol/L)	Automated chemiluminescent assay	Any supplement use was ceased for a minimum of 3 weeks before the blood collection	Spearman correlation coefficients and Pearson correlation coefficients	r_{QM1} = N/A, r_{QM2} = 0.35 for DFE, r_{QR} = 0.01 for dietary folate equivalent (DFE, energy-adjusted)
Jackson et al. (2011), Jamaica	159 men (mean 62 years)	120	Previous year	N/A	N/A	Largely based on the US Department of Agriculture Nutrient Database (2007)	Serum folate (M1, ng/mL) RBC folate (M2, ng/mL)	Competitive immunoassay	Any supplement users were excluded from the study ($n = 8$)	Pearson (crude) and partial correlation coefficients (age, energy, BMI, smoking adjusted)	r_{QM1} = 0.22 (crude), r_{QM2} = 0.11 (crude, NS), r_{QM1} = 0.25 (adjusted), r_{QM2} = 0.33 (adjusted)
Johansson et al. (2010), Sweden	96 men and 99 women from the basic validation study (30–60 years)	84	Previous year	10 × 24HDRs by telephone interview	Equally distributed over the year, covering all weekdays	Food composition database of the National Food Administration (year not specified)	Plasma folate (nmol/L)	Quantaphase II radioassay	Yes (no participants reported multivitamin use)	Spearman correlation coefficients	r_{QM} = 0.24 (men, energy-adjusted), r_{RM} = 0.18 (men, energy-adjusted), r_{QR} = 0.41 (men, crude), r_{QM} = 0.20 (women, enrgy-adjusted), r_{RM} = 0.33 (women, energy-adjusted), r_{QR} = 0.57 (women, crude)
Signorello et al. (2010), USA	125 African Americans, 130 non-Hispanic whites, all non-smokers (40–79 years)	89	Previous year	N/A	N/A	Compiled the data from the 24HDRs conducted within NHANES and CSFII (data updated with 2001–2004 estimates)	Serum folate (ng/mL)	*Lactobacillus casei* microbiological assay	Yes, but not added in the FFQ values	Multivariable adjusted partial correlation coefficients	r_{QM} = 0.19 (crude), r_{QM} = 0.26 (adjusted)
Colic Baric et al. (2009), Croatia	13 men and 63 women, all vegetarians (mean 35 years)	39	Previous month	N/A	N/A	National food composition tables (1990)	Serum folate (M1, nmol/L) RBC folate (M2, nmol/L)	Microparticle enzyme immunoassay (Abbott AxSYM System)	Yes	Pearson correlation coefficients	r_{QM1} = 0.41 (DFE), r_{QM2} = 0.36 (DFE)
Colic Baric et al. (2009), Croatia	99 women (21–87 years)	39	Previous month	N/A	N/A	National food composition tables (1990)	Serum folate (M1, nmol/L) RBC folate (M2, nmol/L)	Microparticle enzyme immunoassay (Abbott AxSYM System)	Yes	Pearson correlation coefficients	r_{QM1} = 0.36 (DFE), r_{QM2} = 0.34 (DFE)

Table 1. Continued

Study	Study participants Number (age range)	FFQ/test method		Reference dietary method		Food composition database	Reference biomarker method		Supplement use considered	Main statistical methods	Main results
		Number of items	Reference period	Method	Duration		Biomarkers used	Biochemical assays used			
Shuaibi et al. (2008), Canada	95 women (18–25 years)	Choice of 91 food pictures (Food Choice Map, FCM)	Usual consumption and frequency over a usual week	A food record (self-administered)	3 days	Canadian Nutrient File (2001b)	Serum folate (ng/mL)	Quantaphase folate radioassay	Yes	Pearson correlation coefficients, Validity coefficient (method of triads)	$r_{QM} = 0.43$ (DFE), $r_{RM} = 0.39$ (DFE), VCQ = 0.97, VCR = 0.79
van de Rest et al. (2007), the Netherlands	1281 men and women (50–75, mean 60 years)	89	Past 3 months	N/A	N/A	Dutch Food Composition Table (2001)	Serum folate (M1, nmol/L) RBC folate (M2, nmol/L)	Chemilumine-scent immunoassay analyzer	Supplement users excluded	Spearman correlation coefficients	$r_{QM1} = 0.14$, $r_{QM2} = 0.05$
Verkleij-Hagoort et al. (2007), the Netherlands	53 women (24–44, median 32 years)	121	Past 4 weeks	3 × 24HDRs by 20 min telephone interview	Three successive weeks (2 weekdays + 1 weekend day)	Dutch Food Composition Table (2001)	Serum folate (M1, nmol/L) RBC folate (M2, nmol/L)	ADVIA 120 hematology analyzer	Supplement users excluded	Pearson correlation coefficients, Validity coefficient (method of triads)	$r_{QM1} = 0.20$, $r_{RM1} = 0.22$, $r_{QM2} = 0.28$, $r_{RM2} = 0.49$, $r_{QR} = 0.98$ (deattenuated); VCQ = 0.94 when serum folate was the biomarker; VCQ = 0.75 when RBC folate was the biomarker
Hickling et al. (2005), Australia	568 men and women (33–83, mean 59 years)	19 with a folate intake tool, FIT	1 week	N/A	N/A	Derived from McCance and Widdowson's The Composition of Foods (1991)	Serum folate (nmol/L)	Automated immunoassay	Yes	Pearson correlation coefficients between serum folate and FIT-A (frequency of consumption), and FIT-B (frequency + serving size)	$r_{QM} = 0.54$ (FIT-A, DFE), $r_{QM} = 0.49$ (FIT-B, DFE)
Shai et al. (2005), Israel	161 men and women (mean 50 years)	126 food groups	N/A	6 × 24HDRs by interview at home	N/A	USDA food composition database (1976–1999) + >2000 Israeli/local foods	Plasma folate (nmol/L)	Microparticle enzyme immunoassay (Abbott AxSYM System)	Yes	Pearson partial correlation coefficients, Validity coefficient (method of triads)	$r_{QM} = 0.47$ (deattenuated), $r_{RM} = 0.41$ (deattenuated), $r_{QR} = 0.45$ (deattenuated), VCQ = 0.72
Drogan et al. (2004), Germany	203 men and 160 women (40–65 years)	148	Previous year	N/A	N/A	German food code and nutrient database (1999)	Plasma folate (M1, nmol/L) RBC folate (M2, nmol/L)	Ion-capture assay kit (IMx Abbott Diagnostics)	Yes but not included in the anlaysis	Pearson correlation coefficients	$r_{QM1} = 0.06$ (DFE, NS), $r_{QM2} = 0.08$ (DFE, NS), $r_{M1M2} = 0.63$

Table 1. Continued

Study	Study participants Number (age range)	FFQ/test method		Reference dietary method		Food composition database	Reference biomarker method		Supplement use considered	Main statistical methods	Main results
		Number of items	Reference period	Method	Duration		Biomarkers used	Biochemical assays used			
Yen et al. (2003), USA	28 women (21–47 years)	7 days of folate-focused 24HDRs by telephone or email	5 weekdays + 2 weekend days	122-item FFQ	Reference period—past month	University of Minnesota Nutrition Database (2000)	Plasma folate (nmol/L)	Immunoassay using direct chemiluminescent technology (Bayer Diagnostics ADVIA Centaur folate assay)	Yes	Spearman correlation coefficients	$r_{QM} = 0.35$ (folate-focused recall, NS), $r_{RM} = -0.26$ (FFQ, NS)
Bacardi-Gascon et al. (2003), Mexico	34 women from middle SES (18–32, mean 25 years)	31	N/A	5-day-weighed food record	3 consecutive working days + 2 consecutive weekend days	First DataBank + Mexican brand label	Serum folate (M1, nmol/L) RBC folate (M2, nmol/L)	Dualcount radioassay using an isotope kit	Supplement users excluded	Pearson correlation coefficients	r_{QM1} not significant, $r_{RM1} = 0.40$ (deattenuated), r_{RM2} not significant, $r_{QR} = 0.71$, $r_{M1M2} = 0.52$
Iso et al. (2003), Japan	87 men (40–69 years)	138	N/A	N/A	N/A	Mostly from the Tables of Food Composition in Japan (2000) + USDA Nutrient Database for Standard Reference (1977)	Plasma folate (nmol/L)	Chemilumine-scent immunoassay	No supplement user	Spearman correlation coefficients	$r_{QM} = 0.26$ (energy-adjusted)
Pufulete et al. (2002), UK	36 men and women (22–65, mean 36 years)	90	Previous year	7-day-weighed food record (7d-WR)	7 consecutive days	McCance and Widdowson's The Composition of Foods (1991)	Serum folate (M1, nmol/L) RBC folate (M2, nmol/L)	Ion-capture assay kit (IMx Abbott Diagnostics)	Yes	Pearson correlation coefficients, Validity coefficient (method of triads)	$r_{QM1} = 0.47$ (crude), $r_{QM2} = 0.25$ (crude, NS), $r_{RM1} = 0.39$ (crude), $r_{RM2} = 0.38$ (crude), $r_{QR} = 0.53$ (partial), $r_{M1M2} = 0.41$ (crude); VCQ = 0.85 (men), VCQ = 0.69 (women), VCR = 0.81 (men), VCR = 0.44 (women) when serum folate was the biomarker; VCQ = 0.69 (men), VCQ = 0.41 (women), VCR = 1.00 (men), VCR = 0.72 (women) when RBC folate was the biomarker
Knutsen et al. (2001), USA	193 nonhispanic men and women-97 black and 96 white (mean 50 years)	200 questions	Previous year	8 × 24HDRs by telephone interview	4 weekdays + 2 Sundays and 2 Friday evening/ Saturday	Nutional Data Systems from University of Minnesota, (1993)	RBC folate	Competitive ligand-binding radioassays	Yes	Pearson correlation coefficients	$r_{QM} = 0.24$ for blacks, $r_{QM} = 0.32$ for whites; $r_{RM} = 0.54$ for blacks (partial), $r_{RM} = 0.55$ for whites (partial) (energyadjusted)

N/A, not available; 24HDRs, 24-h dietary recalls; DFE, dietary folate equivalents; NS, not statistically significant; r_{QM}, correlation coefficient between FFQ and a biomarker; r_{RM}, correlation coefficient between a reference dietary method and a biomarker; r_{QR}, correlation coefficient between FFQ and a reference dietary method; VC, validity coefficient; NHANES, National Health and Nutrition Examination Survey; CSFII, the US Department of Agriculture's Continuing Survey of Food Intakes by Individuals; USDA, US Department of Agriculture.

Table 2. Comparison of different methods used in studies to validate dietary folate intake

Study	Study population	Test method	Reference method		Time frame of study administration	Statistical analysis		Main findings/ conclusions of the study	Discussion
			Dietary method	Biomarkers		Test versus reference dietary method	Test method versus biomarkers		
Fayet et al. (2011), Australia	Women only, primarily white and English-speaking university students	• Electronic, semiquantitative self-administered FFQ with 235 items • Appropriate for young and educated participants	• Only a part of subsample completed 24HDRs (two to three replicates), with limited days covered • Eating habits might have been influenced due to the face-to-face interview • Sources of error in the 24HDRs tend to be correlated with the error in the FFQ	• Blood collection was made a week before the FFQ distribution • No detailed information on the performance of the biochemical assay (e.g., within- and between-run coefficients of variation)	Assessments were completed over relatively close time span each other (within 3 months)	• Energy underreporting evaluated using the Hayter and Harry equation • Paired *t*-test and ANOVA made for comparing means • Energy adjustment made using the residual method • Bland–Altman plot indicated a small dispersion in folate value • Classification into categories of consumption by two different dietary methods (no Kappa statistics)	• Pearson/ Spearman rank correlation coefficient	• Moderate agreement between the two dietary methods was observed • Significant diet-biomarker correlations were observed for folate, folic acid, DFE • The FFQ is valid and useful in ranking individuals based on their nutrient intakes for vitamin B12 and folate	• Highly educated female population; limited generalisability of the results • Single blood collection with no consideration of seasonal variation • No discussion on how blood levels equate to dietary consumption
Jackson et al. (2011), Jamaica	Men only, enrolled from the control group of a case-control study of diet and prostate cancer in Jamaica	• 120-item FFQ, administered by interview by research nurses • Food models, utensils, measuring cups and tapes were used for portion size estimation • Not clear how long the interview requires • May not be ideal in large epidemiological studies	N/A	• It is not clear whether biomarker information was collected on days that were representative of the total frame of the FFQ • No information on the within- and between-run coefficients of variation of the biochemical assay	Time sequence of the FFQ and biomarker administration is not certain	N/A	• *t*-test to assess difference between the means for the lowest and highest quartiles • Pearson correlation coefficient • Multivariable adjusted partial correlation coefficients	• Unadjusted RBC folate was not related to dietary intakes, i.e., biomarkers may not necessarily reflect long-term dietary intake • Serum but not RBC folate increased with increasing levels of dietary intakes	• Limited generalisability (male population) • A single blood collection with no consideration of seasonal variation • Borrowed food composition database values for calculating nutrient content of food items
Johansson et al. (2010), Sweden	Part of the population-based cohort, a representative sample	• Self-administered 84-item semiquantitative FFQ	• Sufficient number of replicate 24HDRs to represent average intake and cover the interval of time corresponding to the FFQ (1 year) • High rate of compliance (79% completed ten interviews)	• Venous blood samples were drawn before the baseline FFQ was completed • The total coefficients of variation (%) for folate were 3.9–6.9% at levels 3.8–21.5 nmol/L	• FFQ was administered before the 1-year period of ten unannounced occasions of 24HDRs • Time frame of FFQ and biomarker measurements do not correspond	• Energy adjustment made using the residual method • Deattenuated correlation coefficients calculated using repeated 24HDRs • Calibration coefficients estimated by linear regression • No Bland–Altman plot used to investigate agreement	• Spearman rank correlation coefficient	• The moderate correlation coefficients found in the study were similar to the other FFQ validation studies • The northern Sweden FFQ is valid for ranking individuals by dietary intake of folate but to a lesser extent for vitamin B12	• A single blood collection with no repeated measure • The association between dietary intakes and biomarkers was investigated only by correlation

Table 2. Continued

Study	Study population	Test method	Reference method		Time frame of study administration	Statistical analysis		Main findings/ conclusions of the study	Discussion
			Dietary method	Biomarkers		Test versus reference dietary method	Test method versus biomarkers		
Signorello et al. (2010), USA	Part of the prospective cohort study comprised primarily of African-American and non-Hispanic white residents of the south-eastern United States	• 89-item FFQ, administered through a computer-assisted in-person interview • Not clear how long the interview requires to complete the FFQ	N/A	• Blood samples were taken at the time of the baseline interview • Approximately 92% provided either blood sample or a buccal cell sample • No information on the within- and between-run coefficients of variation of the biochemical assay	FFQ and blood samples were collected at the time of enrollment, however FFQ reference period was to the year preceding enrollment	N/A	• Multivariable adjusted partial correlation coefficients • Comparison of mean blood values across quintiles and deciles of FFQ intake • Linear regression models	• The increase in blood level of folate appeared to level off after the third or fourth quintile of FFQ-estimated intake • The correlation coefficients between FFQ intakes and serum folate varied by race, sex, educational level, obesity status and vitamin supplement use • Overall the FFQ appears to generate useful dietary exposure rankings in the cohort	• A single blood collection with no repeated measure • No consideration of seasonal variation • Multiple testing with small subgroup analyses
Colic Baric et al. (2009), Croatia	Vegetarians with the majority being female	• A self-administered 39-item folate FFQ with photos • Food items based on Croatian food composition database • Synthetic folic acid consumption/ bioavailability considered	N/A	• No detailed information available on the measurement of serum and RBC folate • It is not clear whether biomarker information was collected on days that were representative of the total frame of the FFQ	Time sequence of the FFQ and biomarker administration is not certain	N/A	• Principal component analysi • Pearson correlation coefficients • Cross-classification into quartiles for folate intake and biomarkers with weighted kappa values	• The correlation coefficients between FFQ intakes and serum and RBC folate did not differ significantl • Pearson's correlation, weighted kappa and classification into quartiles were more favourable in vegetarians than among omnivore women in the previous validation study (Colic Baric et al., 2009, see below) • The folate FFQ is valid for measuring dietary folate equivalent intake in Croatian vegetarians	• Limited generalisability (vegetarians) • Food composition databases used was outdated • Not certain how much the selected 39 food items contribute to total dietary folate intake • A single blood collection with no consideration of seasonal variation • No detailed information on the performance of the biochemical assay
Colic Baric et al. (2009), Croatia	Women only, recruited from the university community	• A self-administered 39-item folate FFQ with photos • Food items based on Croatian food composition database • Synthetic folic acid consumption/ bioavailability considered	N/A	• No detailed information available on the measurements of serum and RBC folate • It is not clear whether biomarker information was collected on days that were representative of the total frame of the FFQ	Time sequence of the FFQ and biomarker administration is not certain	N/A	• Principal component analysis (PCA) • Pearson correlation coefficient • Cross-classification into quartiles for folate intake and biomarkers with weighted kappa values	• The correlation coefficients between FFQ intakes and serum and RBC folate did not differ significantly • Use of folate status factor from PCA resulted in a higher correlation with dietary folate intake compared with single biomarkers • The folate FFQ is valid for measuring dietary folate equivalent intake in adult Croatian women	• Limited generalisability (women only) • Food composition databases used was outdated • Not certain how much the selected 39 food items contribute to total dietary folate intake • A single blood collection with no consideration of seasonal variation • No detailed information on the performance of the biochemical assay

Table 2. Continued

Study	Study population	Test method	Reference method		Time frame of study administration	Statistical analysis		Main findings/ conclusions of the study	Discussion
			Dietary method	Biomarkers		Test versus reference dietary method	Test method versus biomarkers		
Shuaibi et al. (2008), Canada	Women of childbearing age recruited from the university	• FCM administered by an in-person interview • Relatively low participant burden • Automated data entry • Portion size estimation was aided by photos • FCM interview requires 50 min, and may not be ideal for large-scale studies	• Self-administered 3-day food record was given after the FCM interview • Records were reviewed with each participant to ensure completeness • Sources of error in the 3-day food record and in FCM were thought to be less correlated due to differences in methodologies	• The accuracy of the radioassay methods was checked through serial replication of three levels of control sera • It is not clear whether biomarker information was collected on days that were representative of the total frame of the FFQ	Time sequence of the FCM and biomarker administration (clinic visit) is not clear	• Energy adjustment for the intake using the residual method • Classification into categories of consumption by two different dietary methods (no Kappa statistics) • No Bland–Altman plot used to investigate agreement	• Pearson correlation coefficient (no adjustment) • Validity coefficient calculated using the method of triads	• No significant differences between the two dietary methods in the correlations with serum values • The validity coefficient for the FCM was higher than that for the 3-day food record • Food composition database was updated for the folate value using DFE • For estimating folate intake in women in reproductive age, the FCM provides valid results	• Limited generalisability • A single blood collection with no consideration of seasonal variation • Correlations between FCM and the 3-day food record were not energy-adjusted • The error correlation assumption for validity coefficient may not be valid
van de Rest et al. (2007), the Netherlands	Elderly population participating in the three different folic acid supplementation intervention studies	• FFQ developed to measure folate • 87 selected items cover at least 80% of the average folate intake • Second FFQ was administered in a subsample of the participants (3 years of interval; changes in diet are likely) • Updated folate content database was used	N/A	• Blood samples were taken at the start of the intervention period of each study • RBC folate in a subsample was measured in duplicate • No information on the within- and between-run coefficients of variation of the biochemical assay	Assessments were completed over relatively close time span each other (within 3 months)	N/A	• ANOVA for mean differences • Spearman correlation coefficients • Linear regression to determine the best predictors of folate concentration • Comparison of mean blood values across quartiles of FFQ intake	• Serum and not RBC folate concentrations correlated positively with folate intake (correlations weaker than in other studies) • FFQ could rank participants according to folate intake • Supplement users excluded and folic acid fortification was not allowed in the country; unlikely to have effect on biomarker levels • Duplicated measures of RBC folate in a subsample did not improve correlations	• Data came from elderly participants of intervention studies, hence limited generalisability of the results • High folate consumers were excluded due to the exclusion criteria of the study, total homocysteine levels <26 μmol/L • No consideration of seasonal variation
Verkleij-Hagoort et al. (2007), the Netherlands	Women of reproductive age recruited from an ongoing case-control study	• Self-administered FFQ • 121 items covered the daily intake of each nutrient for at least 90% of the population mean intake	• Three sets of 24HDR were collected by telephone interview • Sources of error in the 24HDRs may be correlated with the error in the FFQ	• All samples were analysed within 3 months after collection • Hemolysate folate concentration was recalculated into RBC folate concentration using the formula	Assessments were completed over relatively close time span each other (within 2 months)	• Energy underreporting was evaluated using the Goldberg method • Energy adjustment for the correlation using the residual method • Paired *t*-test and for comparing means • Pearson correlation coefficients • Deattenuated correlation coefficients using repeated 24HDRs • No Bland–Altman plot, no cross-classification and no Kappa statistics used	• Pearson correlation coefficient (no adjustment) • Validity coefficient calculated using the method of triads	• The correlation coefficients between the FFQ and the 24HDR folate were comparable with other studies • The correlation between the FFQ and the biomarkers is higher for RBC than serum folate • The validation coefficients were high for folate and greater than 1 for vitamin B12 • The adapted FFQ is a reliable tool to estimate dietary intake of other nutrients including folate	• Small sample size • Women of reproductive age, limited generalisability of the results • Single blood collection with no consideration of seasonal variation • The error correlation assumption for validity coefficient may not be valid

Table 2. Continued

Study	Study population	Test method	Reference method		Time frame of study administration	Statistical analysis		Main findings/ conclusions of the study	Discussion
			Dietary method	Biomarkers		Test versus reference dietary method	Test method versus biomarkers		
Hickling et al. (2005), Australia	Subsample of a follow-up cohort study	• Self-administered Folate intake tool (FIT) including 19 folate-related key items • FIT-A to indicate consumption frequency only • FIT-B to indicate serving size + frequency • Very rapid to both complete and analyse (<5 min) • Repeatability was tested using a second set of FIT (n = 277)	N/A	• Not clear whether biomarker information was collected on days that were representative of the total frame of the FIT • Limited information on the procedures of biochemical analysis • Interassay coefficient of variation was 5.5%	Whether assessments were completed over relatively close time span not certain	N/A	• Pearson correlation coefficients	• FIT administration showed low respondent burden, ease of administration and low cost • Collecting serving size information did not improve the measurement of nutrient intake • FIT should provide a practical tool for assessing folate intake	• Food items that contributed ≥20 μg folate per serving were included, however it is not certain how much the selected food items contribute to total dietary folate intake • A single blood collection with no consideration of seasonal variation • Poor statistical methods: validity and reliability were tested by Pearson correlation coefficients
Shai et al. (2005), Israel	Participants were likely to be men, highly educated and healthy, from the Dietary Evaluation and Attenuation of Relative Risks study in Israel	• Self-administered FFQ with 126 food groups that were main contributors to the between-person variation of each nutrient • Three repeated FFQs were collected over 13 months • Reference period not reported	• Six sets of interview-based 24HDRs were collected on random workdays over the same 13 months of study period	• Blood samples were collected twice over the same 13 months of study period • Quality control systems were used • No detailed information on the performance of the biochemical assay	Assessments were completed over the same time span (13 months of study period) however the FFQ reference period was not reported	• Seasonality was considered and adjusted • Energy adjustment using the residual method • Energy underreporting not evaluated • Deattenuated correlation coefficients calculated using repeated 24HDRs • No Bland–Altman plot, no cross-classification and no Kappa statistics used	• Multivariable adjusted partial correlation coefficients • Validity coefficient calculated using the method of triads	• Inclusion of vitamin supplements did not improve the correlations of the biomarkers to the questionnaires • FFQs performed better than the 24HDRs and the biomarkers in estimating true intake • FFQ is a valid and reproducible instrument for assessing dietary intake	• Limited generalisability (highly educated and healthy) • The method of triads relies on the assumption that errors in two dietary methods are not correlated which may not be valid
Drogan et al. (2004), Germany	Randomly chosen subsample of the EPIC-Potsdam cohort	• Self-administered 148 item FFQ with questions on regular supplement use	N/A	• Within-/between-run coefficients of variation (%) were 3/6 for plasma folate and 6/9 for RBC folate	Time sequence of the FFQ, 24HDRs and biomarker administration is not certain	N/A	• t-test to assess difference between men and women • Pearson correlation coefficients	• Study was aimed to compare RBC folate and plasma folate in relation to dietary folate intake • RBC folate and plasma folate were significantly correlated with each other • DFE from the FFQ was only very weakly associated with blood folates	• Single blood collection with no consideration of seasonal variation • Not clear whether food composition database was updated with folate values • Insufficient evidence to conclude that plasma folate could be used as a marker of folate status in large-scale epidemiological studies

Table 2. Continued

Study	Study population	Test method	Reference method		Time frame of study administration	Statistical analysis		Main findings/ conclusions of the study	Discussion
			Dietary method	Biomarkers		Test versus reference dietary method	Test method versus biomarkers		
Yen et al. (2003), USA	Premenopausal women recruited from the university community	• Telephone-/email-based folate-focused 24HDR (by using folate-specific food list) was collected for a 7-day period • Each contact requires 5–10 min	• After the 7 × 24HDRs, participants were asked to complete the FFQ with reference period of past month	• Blood samples were collected during the initial clinic visit • No information on the within- and between-run coefficients of variation of the biochemical assay	Assessments were completed over close time span each other (within 1 month)	• Energy underreporting not evaluated • Energy adjustment was made only for the reference FFQ method, not for the test method • Cross-classification into tertiles of consumption by two different dietary methods (no Kappa statistics) • No Bland–Altman plot used to investigate agreement	• Spearman rank correlation coefficients • Multivariate analysis of variance using plasma folate as the dependent variable and dietary intake as the independent variable	• Focused recall and the FFQ demonstrated 67% concordance in the highest and lowest tertile ranking for folate intake inclusive of supplements • Folate intake estimated from the focused recall approach was marginally significantly correlated with plasma folate concentration	• Limited sample size, generalisability (highly educated and young, willing participants) • Single blood collection with no consideration of seasonal variation • Possibility of change in dietary habit or misreporting due to provision of information on the study aims and training in the use of the instruments • Not clear how the items in folate-specific food descriptor were selected and how much they contribute to total dietary folate intake
Bacardi-Gascon et al. (2003), Mexico	Mexican women of reproductive age, representing middle socioeconomic status (SES)	• FFQ was self-administered 3 months after completing 5-day-weighed food record (5d-WR) and blood sample collection • FFQ reference time period not reported • Portion size estimation was aided by food replicas	• 5d-WR was collected from the participants from the middle SES	• Blood samples were collected during the initial clinic visit after completing a 5d-WR • No information on the within- and between-run coefficients of variation of the biochemical assay	Administration of blood sample collection, 5d-WR and FFQ had 6 months of time gap however the FFQ reference period was not reported	• Energy underreporting not evaluated • Energy adjustment was not made • Cross-classification into tertiles of consumption by two different dietary methods (no Kappa statistics) • No Bland–Altman plot used to investigate agreement	• Pearson correlation coefficients • Correlations were corrected for the intraindividual variance by calculating the ratio of intra- to inter-individual variance for average folate intake • Cross-classification of folate level by 5d-WR and blood level tested by chi-square goodness-of-fit	• 33% of participants had low levels of serum and RBC folate • No statistically significant correlations between folate intake and RBC folate	• Limited sample size, generalisability (volunteers from a single industry) • Single blood collection with no consideration of seasonal variation • Limited information on the food composition database used • Radioassay may not be an ideal method for RBC folate analysis • Validity of the dietary instruments was tested only by Pearson correlation coefficients
Iso et al. (2003), Japan	A male subsample of the participants in a cohort study on cancer and cardiovascular diseases	• FFQ was self-administered 6 months to 1 year after blood collection • FFQ methods were not described in detail • Reference period not shown	N/A	• Blood samples were collected twice in winter and summer during the initial clinic visit • No information on the within- and between-run coefficients of variation of the biochemical assay	Assessments were completed over 1 year of time span however FFQ reference period not reported	N/A	• Energy underreporting not evaluated • Energy adjustment for the intake data made using the residual method • Spearman correlation coefficients • Comparison of mean dietary values across quintiles of plasma concentration	• Moderate association between dietary folate and plasma folate • A steady increase in mean folate intake from the lowest to the highest quintile of plasma folate was observed	• Participants characteristics were not shown • Seasonal variation was considered by using two sets of blood collection, however it was not analysed separately in relation to dietary intake • No sufficient evidence to conclude the FFQ could reasonably rank individuals • Validity of the dietary instruments was tested only by Spearman correlation coefficients

Table 2. Continued

Study	Study population	Test method	Reference method		Time frame of study administration	Statistical analysis		Main findings/ conclusions of the study	Discussion
			Dietary method	Biomarkers		Test versus reference dietary method	Test method versus biomarkers		
Pufulete et al. (2002), UK	Participants recruited from the university community	• FFQ developed to assess consumption of major sources of folate • Food items that contributed more than 10% of the folate intake among subjects in the top fifth of folate intake were selected to be included • FFQ was self-administered at the clinic visit after blood draw • Portion size estimation was aided by photos	• 7-day weighed food record (7d-WR) was completed within 10 days of the clinic visit	• Blood samples were collected during the initial clinic visit • No information on the within- and between-run coefficients of variation of the biochemical assay	Assessments were completed over close time span each other (within 1 month), however FFQ reference period was a previous year	• Energy underreporting from the 7d-WR was evaluated using the Goldberg method • Energy adjustment was made only for 7d-WR, not for the test method • A paired *t*-test for comparing means • Cross-classification into tertiles of consumption by two different dietary methods (no Kappa statistics) • No Bland–Altman plot used to investigate agreement	• Pearson correlation coefficients • Validity coefficient calculated using the method of triads	• Folate intakes were significantly higher on the FFQ than on the 7d-WR in women • Folate intakes estimated by FFQ were significantly correlated with serum but not with RBC folate • The strength of the association was greater in men than in women • Validity coefficients were higher for the FFQ than for the 7d-WR when serum folate was used as the biomarker but lower when RBC folate was the biomarker	• Limited sample size, generalisability (educated volunteers) • Single blood collection with no consideration of seasonal variation • Agreement was not statistically tested • Updated food composition database is necessary • The error correlation assumption for validity coefficient may not be valid
Knutsen et al. (2001), USA	Random sample of a new cohort of 7th-day adventists	• FFQ was self-administered after completion of the first four 24HDRs and before the additional four 24HDRs	• Two sets of 4 × 24HDRs were collected by telephone interview over 6 months with each set covering 2 weekdays, 1 Friday evening/Saturday and 1 Sunday • Interviews were double checked for quality control	• Blood samples were collected during the clinic visit • Not clear whether biomarker information was collected on days that were representative of the total frame of the dietary assessment • No information on the within- and between-run coefficients of variation of the biochemical assay	Time sequence of the dietary measurements and biomarker administration is not certain	• Energy underreporting not evaluated • Energy adjustment for the intake using the residual method • Deattenuated correlation coefficients (adjusting only for within-person error) calculated using repeated 24HDRs • No Bland–Altman plot used to investigate agreement • No cross-classification and no Kappa statistics	• Pearson correlation coefficients	• Moderately high correlations between 24HDR folate and RBC folate • FFQ folate was less well correlated with RBC folate • Low and non-significant correlation with the FFQ among blacks who did not use supplements	• Limited generalisability (highly educated 7-day adventists) • A single blood collection with no consideration of seasonal variation • Validity of the dietary instruments was tested only by Pearson correlation coefficients

N/A, not available; 24HDRs, 24-h dietary recalls; DFE, dietary folate equivalents; NS, not statistically significant; VC, validity coefficient; WR, weighed food record.

complete for each participant [38] while the focused recall administered in young women either by telephone or e-mail required 5–10 min [42]. The reproducibility of the test instruments was reported in some studies where the same test instruments were administered on the same participants at least twice [31, 33, 36, 37, 40].

3.2.4 Food composition database

All studies indicated that dietary folate intake was calculated with different software programmes based on various food composition databases (Table 1). While the majority used locally available food composition databases, some applied data from other countries with adding regionally obtainable information [26, 31–33, 37]. It was not always clearly stated when the folate content was last updated in the database, especially with regard to recently introduced folic acid-fortified foods and dietary supplements containing folic acid [27–29, 31, 36–38, 40, 41].

3.2.5 Dietary supplement use

Quite a few studies collected information on dietary supplements and considered them in the analysis [27, 28, 31, 34–38, 42]. Some studies excluded any dietary supplement users or asked participants to cease any supplement use before the blood collection [26, 30, 33, 40, 41]. Altogether, eight of 17 studies did not take dietary supplement use into account in their analyses [26, 29, 30, 32, 33, 39–41].

3.2.6 Mode of folate expression

Most studies expressed daily dietary folate values in micrograms. Several studies used Dietary Folate Equivalents (DFE) justified by inequities in bioavailability between folic acid and naturally occurring food folate [27–30]. Except for one study where DFE was not clearly defined [30], studies calculated the DFE values according to the procedure suggested by the US Institute of Medicine, i.e., 1 DFE is regarded as being equivalent to 1 μg of folate from the diet, 0.6 μg folic acid from fortified foods and 0.5 μg folic acid from supplements [44, 45].

3.3 Test versus reference dietary method

Nine of 17 studies [26, 30, 34–38, 41, 42] evaluated the validity of one dietary instrument against both dietary reference method and biomarkers, while eight studies [27–29, 31–33, 39, 40] assessed validity of a dietary method only against biomarkers. We explored in this section the former nine studies that compared the test instrument against the dietary reference method (i.e., relative validity) additionally to the comparison between the test instrument and biomarkers.

3.3.1 Types of reference dietary methods

The most commonly chosen dietary reference method among those nine studies was 24HDRs. The 24HDRs were repeated at least twice [30] to maximum ten times [34], administered mostly by a telephone interview. Few studies chose other self-administered methods such as a 3-day food record [38], a 5-day weighed food record [26] and a 7-day weighed food record [36].

3.3.2 Time frame of the test instrument and the dietary reference method administration

As suggested by Cade and colleagues in their review that provides guidance on the development, validation and use of FFQs [46], the test instrument should be administered prior to the assessment of the reference measure. In addition, the test instrument and the reference method should assess diet over the same time span [46]. Only few studies reported to have their test and reference dietary assessments conducted within the time period of 1–3 months [26, 30, 42] and 1 year [34]. In some studies, the reference time period of the test instrument did not correspond to one of the reference methods that covered a shorter time period [35, 36], or time period was simply not reported [26, 37] even though both methods were actually administered within a relatively short period of time.

3.3.3 Statistical methods (test versus reference dietary method)

Under reporters for energy intake were considered for the analysis in only few studies [30, 36, 41]. Most studies that used general FFQs were able to adjust for energy intake [30, 34, 35, 37, 38, 41] while it was not possible for studies that used folate-focused instruments [36, 42] to consider energy adjustment when comparing the test instrument with the reference dietary method.

The predominantly used statistical method to compare the test instrument and the dietary reference method assessing folate intake was the correlation coefficient as shown in Tables 1 and 2. The use of Bland–Altman plot (graphs of the differences between the test and reference measurements against the mean of the two [47] with limits of agreement calculated as the mean difference plus and minus two standard deviations [48]) has been recommended when investigating validity as it assesses the agreement graphically between the methods across the range of intakes [46, 49]. However, in this review, only one study [30] reported a small dispersion in folate values between the two dietary methods using the Bland–Altman method (Table 2).

When the correlation coefficients were used, some studies were able to report deattenuated correlation coefficients considering for both intra- and inter-individual

variance using the repeated reference dietary method [34, 37, 41] while one study reported deattenuated correlation coefficients adjusting for random intra-individual variance [35].

Participants were often classified into categories of folate intake by the test instrument and the reference method, and the percentage of participants was calculated that correctly fell into the same category or were misclassified into the opposite category (Table 2). In this case, additional use of the Kappa or weighted Kappa statistics, which test the degree of agreement between folate intakes measured by the test and the reference dietary method in the predefined categories [50, 51] would be informative [46, 52], but none of the studies used this approach for the cross-classification.

3.4 Test instruments versus reference biomarkers

3.4.1 Types of reference biomarkers

The most frequently chosen biomarker (94%) was serum or plasma folate [26–34, 36–42]. More than half of the studies used both serum or plasma and RBC folate [26–30, 33, 36, 40, 41]. Biochemical assays used in studies include automated immunoassay, radioassay method, and microbiological assay (Table 1). However, information on the within- and between-run coefficients of variation of the biochemical assay used was often lacking and only few studies gave a detailed description of the assay performance [29, 31, 34].

3.4.2 Time frame of the test instrument and the reference biomarker administration

It is important that the biomarker information is collected on days that are representative of time period of the test instrument [46]. However, the majority of studies did not clearly report time period for administration of the test instrument, reference dietary method and for the blood sampling. Even though the test instruments and reference biomarkers in some studies were administered within a relatively short period of time, quite often the time period of the test instrument was unknown [26, 32, 37] or the year preceding enrolment [34, 36, 39] and therefore prior to blood collection. Only few studies assessed dietary and blood level of folate within a relatively short time span [30, 40, 42].

None of the studies made repeated blood collections with consideration of seasonal variation, except the study by Iso et al. [32] that collected blood samples twice, both in winter and summer. However, dietary intake was assessed only once by FFQ without special consideration of seasonality; therefore analyses of the intake in relation to blood levels across seasons were not made [32].

3.4.3 Statistical methods (test instrument versus reference biomarkers)

In terms of the statistical analyses, correlation coefficients were the main methods chosen in all studies as shown in Tables 1 and 2. When the data were not normally distributed, studies either applied Spearman rank correlation coefficients [30, 34, 40] or Pearson correlation coefficients after the variables were logarithmically transformed [26, 29, 35, 37, 38, 41]. Correlations were deattenuated [26, 35, 37, 41], energy-adjusted [30, 32, 35] or multivariable adjusted [33, 39]. Other methods include linear regression [39, 40], comparison of biomarker values across categories of FFQ intake [27, 28, 32, 39, 40], principal component analysis [27, 28], validity coefficient using the method of triads [36–38, 41] and use of *t*-test or analysis of variance for tests of differences in means [29, 40].

3.5 Principal findings

The correlation coefficients between different instruments varied greatly (Table 1). The correlations between folate intake based on the test instrument and serum/plasma folate concentrations ranged from 0.06 [29] to 0.54 [31] with median values of 0.35 from five studies in women, 0.25 from three studies in men and 0.41 from seven studies in men and women combined. The correlations between folate intake based on the test instrument and RBC folate concentration ranged from 0.05 [40] to 0.36 [28], with median values of 0.34 from three studies in women, 0.25 from five studies in men and women combined and 0.33 in one study in men [33]. Three studies that examined both plasma or serum folate and RBC folate concentrations, reported the correlations between the two biomarkers as 0.41 [36], 0.52 [26] and 0.63 [29], respectively.

The correlations between folate intake based on the test instrument and the reference dietary method, on the other hand, showed a range from 0.01 [30] to 0.98 [41] with median values of 0.64 from four studies in women, 0.49 from two studies in men and women combined and 0.41 in one study in men [33].

Overall, folate intake assessed by the test instrument and the blood folate concentration were statistically significant, but relatively moderately correlated while it showed higher correlation with the reference dietary method [26, 34, 36, 41]. Studies conducted in women generally showed higher correlations than the one study conducted only in men. For both men and women, correlations for the test instrument were better with plasma or serum folate than with RBC folate. Correlation coefficients with biomarkers were neither shown to be particularly related to the reference period of the test instrument nor with the number of food items included in the test instrument (Supporting Information Figure S1). However, we noted that the data from the test instrument and the

reference method tended to correlate better when dietary supplement use was included in the analyses [27,28,31,34–38,42].

There were studies that reported the validity coefficients of folate intake measured by the test instrument, estimated from a triangular comparison between questionnaire, reference and biomarker measurements with the method of triads [53]. The validity coefficients of folate intake based on the test instrument and the unknown 'true intake' were 0.97 with the FCM [38], 0.94 with the 121-item FFQ [41], 0.72 with the 126-food group FFQ [37] and 0.85 for men and 0.69 for women with the 90-item folate FFQ [36], when plasma or serum folate concentration was used as biomarkers. When both plasma/serum and RBC folate levels were available, the validation coefficients for the test instrument (FFQ) were higher when plasma/serum folate was the biomarker than when RBC folate was the biomarker.

4 Discussion

We examined 17 published studies that assessed validation of dietary folate intake against folate concentration biomarkers, published between 2001 and 2011. In this review, we observed that the majority used self-reported FFQ to ascertain dietary folate intake and to be validated against biomarkers while 24HDR was often chosen as a reference dietary method. Correlation coefficients were the most frequently used statistical measure in the studies reviewed. Correlations between folate intake assessed by the test instrument and the blood folate concentration were statistically significant, but the strength varied from weak to moderate ($r = 0.05$–0.54). These validation studies varied greatly in terms of study characteristics, such as the study population, sample size, the procedure of data collection, the consideration of dietary supplement use, the procedure of biochemical analysis and the time frame of study administration.

Studies often recruited participants on a voluntary basis within a confined study setting, e.g., university community, with a limited number being involved. Participants who volunteer to take part in studies are considered to be self-selected, and may therefore have different characteristics in responding to FFQs than non-volunteers [18, 46]. In addition, the majority of the included participants were highly educated and relatively young. Because participants with higher educational level tended to report higher folate intake [11], it is not possible to rule out the potential that different estimates would have been observed in other populations. This has little implication on the internal validity of the studies, nonetheless care should be taken when generalising these results to other contexts.

Furthermore, the small sample size that was seen in a few studies may have resulted in the limited statistical power for their analyses. For example, studies conducted among 28 women [42] and 34 women [26] had non-significant correlation coefficients, which may have been due to inadequate study power. We observed that the vast majority of the FFQ used as a test instrument were self-administered and incompleteness can therefore also be a substantial source of errors [46]. The completeness of the test instrument was seldom checked or was not reported, in contrast to the reference dietary method that was often checked for completeness by interviewers or study administrators.

In validation studies that compare the test instrument with different reference methods, it is crucial that they measure similar parameters over the same time span [16, 18, 46]. At the same time, recording of dietary data must not interfere with participants' usual dietary habits that may lead to correlated errors in reference and test instruments [54]. However, in many studies that we reviewed, information on the time period of assessment was often lacking. Moreover, reference biomarker measures that are based on a single blood sample and without consideration of within-individual variability may not reflect the blood concentration during the total time frame of the FFQs. This can have even more profound impact if there is a seasonal variation in the folate intake. Vegetables are a major source of folate in the diet and there is some seasonal variation in the consumption of vegetables [55, 56]. Although seasonal variation in folate content of foods appeared to be low due to wide availability of the food items throughout the year [57], it should be pointed out that there were some variations observed in actual dietary folate intake from food sources especially in southern European countries with higher intake being reported in spring and winter [11].

The issues related to the choice of a test instrument need careful consideration. We found a few studies that tested folate-focused instruments against biomarkers [27, 28, 31, 36, 40, 42]. These instruments can be efficient, rapid, and cost-effective in folate intake assessment. However, when using that approach, it is neither possible to adjust for energy intake nor to consider under-reporting as these instruments list only foods rich in folate. Besides, the long list of folate-rich food items may result in overestimation of folate intake as in the case of fruit and vegetable consumption [58]. Thus, the advantages and disadvantages of the use of folate-specific methods need to be taken into account when diet–disease relationships are investigated.

Another important point that needs cautious attention is the use of food composition databases for calculation of folate intake. Previous inventories found that there was lack of agreement in folate databases especially in quantification methods, definitions, mode of expressions, analytical methods and terminologies used across different countries [59,60]. In this review, we have noted that some studies used data from multiple sources that are not necessarily comparable even within the same table. Moreover, some studies employed relatively old data that referred to more than 15 years before the study time period. These data may well be based on outmoded analytical techniques for folate calculation.

Additionally, not all studies reported whether the food composition data appropriately reflected the nutrient contribution from folic acid fortified food products especially in countries where mandatory or voluntary fortification was

introduced [30, 35]. This review also showed that half of the studies did not consider dietary supplement use in their analyses. Failure to include these different sources may lead to an underestimation of overall folate consumption, notable misclassification of individuals regarding the total folate intake and obscure the true relationship between dietary and blood level of folate [61–63].

Few studies discussed appropriateness of the particular biomarkers as reference measures of dietary intake. As pointed out before, the most important question is the association of the biological marker with actual dietary exposure [46, 64]. Studies that we reviewed did not always consider to what extent the concentration biomarker actually reflects dietary folate exposure. The evidence that serum/plasma folate and RBC folate concentrations are responsive to intakes of either natural food folate [65, 66] or supplements and/or fortified food [67–70] has been available from a few intervention studies. Furthermore, the dose–response relationship with folic acid supplementation appeared to be linear for serum as well as RBC folate [71]. However, the magnitude of the effect and the time required to reach steady state concentrations of serum or RBC folate by dietary folate intervention varied in those studies.

Several other explanations exist for the discrepancies found in results of the validation studies apart from various study design-related aspects. Intervening factors such as personal lifestyle characteristics as well as physiological factors can influence the level of concentration biomarkers, making the quantitative relation between dietary intake and biomarker substantially different between individuals [20, 72]. Different features of RBC folate and serum or plasma folate as indicators of folate status should also be considered. While RBC folate is considered to reflect average concentration over the erythrocyte life span (about 120 days) and therefore longer term folate status, folate concentration in serum or plasma is a responsive indicator of more recent folate intake [29, 73]. Intuitively, one would think that RBC folate therefore would show higher correlations with dietary intake assessed over a longer period. Surprisingly, in our review, we observed that correlations for the test instrument were slightly better when plasma or serum folate was the biomarker compared with the ones when RBC folate was the biomarker. This may be attributable in part to the fact that the test instrument, e.g., FFQ, often asked about consumption over the previous 12 months, whereas in reality, it may have reflected more recent consumption. It may also be that the erythropoiesis process itself determines the folate concentration in RBC, a process that is dependent on environmental factors such as erythropoietin production and the availability of iron, vitamin B12 and zinc [74].

Both analytical variability and preanalytical factors may attenuate statistical estimates in the studies. It has been observed that the analysis of serum samples with microbiological methods or radioassay yielded similar folate concentrations, while different results were obtained for RBC folate concentrations [75]. The precision of methods for whole blood folate is usually lower than that of methods that measure folate in serum or plasma [76, 77]. This might be related to the fact that folate in RBC is present as polyglutamates, and complete deconjugation is required for determination of folate concentration [78]. Large between-laboratory differences have been reported for folate measurement [79], attributable to different analytical technologies but also to preanalytical factors. Folate species are unstable compounds that are degraded during sample handling and storage even in frozen samples [80]. Substantial degradation is observed during prolonged storage, at high temperature, in the presence of EDTA, and in the absence of stabilizer, like ascorbic acid [80, 81]. The use of certified reference material, pooled control samples, (partial) duplicate analyses and participation in ring testing is crucial for obtaining reliable folate concentration data.

We found that correlations between reported intake and biomarker measures were higher in studies when dietary supplement use was taken into account in the analysis. The reason for this may be that supplement use substantially contributes to the total amount of folate intake [82]. Supplement use also expands the range of its biomarker measures partly because bioavailability of folic acid is superior to that of naturally occurring food folate [73]. A few studies calculated DFE values adjusting for these differences in bioavailability based on the assumption that the bioavailability of folate from food is 50% lower than that of folic acid [44, 45]. However, the exact relative bioavailability is uncertain, in particular with regards to mixed diets [12, 57, 59, 73, 83]. DFE values should therefore be used with caution as they may introduce imprecision in food composition databases and may potentially lead to misclassification of intake information [59].

Biomarkers are objective measurements that can be used as a surrogate for actual dietary intake, and the precision and accuracy of the estimates are independent of the participants' ability to report foods consumed [20]. However, concentration biomarkers only allow discrimination between substantial differences in intake level, and may therefore serve as a tool to evaluate if a test instrument is suitable for ranking persons according to their consumption [18, 40]. As previously discussed [23, 46], and as we also found in the studies included in this review, that validity was mostly inferred from statistically significant correlation coefficients between the test instrument and the concentration biomarkers. However, caution needs to be exercised as these concentration biomarkers cannot be translated into absolute intakes on a valid scale, i.e., they cannot provide valid reference measurements [20, 23].

More recently, a few dietary validation studies considered the method of triads [53, 84]. This method assumes that the measurements are linearly related to true intake and have independent random errors [53]. However, these assumptions may not be fully met in most cases. Questionnaires and the reference measurements may have some common sources of error even though the random errors of the biochemical marker data are independent of those of two dietary measurements [53]. Violation of the underlying model

assumptions may cause the occurrence of Heywood cases (estimated validity coefficient > 1), which further complicate interpretation of the results [53, 85]. The estimates should therefore be prudently interpreted considering the possibility of biased estimates of validity coefficients related to violation of those assumptions.

It has been suggested that checking agreement between a measurement and its reference measure should be a mandatory step when investigating validity [49]. Agreement can be graphically assessed by the Bland–Altman plots. These plots can also be used when the dietary and biomarker measurements are estimated on different scales, with a conversion factor recalibrating the two variables [86]. The degree of agreement between the measurements in the pre-defined categories can be tested by Kappa or weighted Kappa statistics [50]. Although these statistical methods can provide further information on the acceptable levels of bias and limits of agreement between the measurements and therefore have been encouraged to be applied in validation studies [46], we found that the application of these methods was still not common. Nonetheless, more discussions and development on the appropriate statistical methods for comparing various dietary instruments and concentration biomarkers are required.

This review of the published studies on validity of dietary folate measurements against folate concentration biomarkers identified a number of issues that need to be considered in future studies that intend to validate dietary folate intake against blood folate concentration. These include: (1) completeness of data collection should be checked and reported; (2) appropriate food composition databases with updated information on folate values derived from reliable and validated chemical analysis should be used; (3) the contribution from fortified food items as well as from dietary supplements should be taken into account; (4) depending on the seasonal and regional variations in consumption of folate-containing food items, repeated measurements for both dietary and biomarker are encouraged; (5) biomarker information should be collected on (multiple) days that are representative of the time period of the test instrument; (6) care should be given on different features of RBC folate and serum or plasma folate as indicators of folate status as well as the accuracy of various analytical methods; (7) quality assurance of laboratory methods should be closely monitored and reported; (8) sample size should be carefully considered; (9) statistical analysis should comprise more than merely a correlation coefficient and (10) appropriateness of the use of concentration biomarkers as a reference measure of dietary intake should always be considered carefully. Given the increasing recognition of the role of folate in several diseases, biomarkers of folate can be used as independent or complementary measures that can greatly strengthen the investigation of diet–disease relationships [87], in addition to being used in validation studies. However, as outlined in this paper, before making inferences about validity, care should be taken of methodological issues when comparing dietary instruments with concentration biomarkers.

This work was undertaken during the tenure of a Postdoctoral Fellowship from the International Agency for Research on Cancer, partially supported by the European Commission FP7 Marie Curie Actions—People—Co-funding of regional, national and international programmes (J. Y. P).

The authors have declared no conflict of interest.

5 References

[1] Kim, Y. I., Folate and DNA methylation: a mechanistic link between folate deficiency and colorectal cancer? *Cancer Epidemiol. Biomarkers Prev.* 2004, *13*, 511–519.

[2] Carmel, R., Nutritional anemias and the elderly. *Semin. Hematol.* 2008, *45*, 225–234.

[3] Stanger, O., Fowler, B., Piertzik, K., Huemer, M. et al., Homocysteine, folate and vitamin B12 in neuropsychiatric diseases: review and treatment recommendations. *Expert. Rev. Neurotherapeutics.* 2009, *9*, 1393–1412.

[4] Wolff, T., Witkop, C. T., Miller, T., Syed, S. B., Folic acid supplementation for the prevention of neural tube defects: an update of the evidence for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* 2009, *150*, 632–639.

[5] Homocysteine Studies Collaboration, Homocysteine and risk of ischemic heart disease and stroke. *J. Am. Med. Assoc.* 2002, *288*, 2015–2022.

[6] Ntaios, G., Savopoulos, C., Grekas, D., Hatzitolios, A., The controversial role of B-vitamins in cardiovascular risk: an update. *Arch. Cardiovasc. Dis.* 2009, *102*, 847–854.

[7] Boushey, C. J., Beresford, S. A. A., Omenn, G. S., Motulsky, A. G, A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *J. Am. Med. Assoc.* 1995, *274*, 1049–1057.

[8] Blount, B. C., Mack, M. M., Wehr, C. M., MacGregor, J. T. et al., Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc. Natl. Acad. Sci. USA* 1997, *94*, 5923–0923.

[9] Kim, Y. I., Folate: a magic bullet or a double edged sword for colorectal cancer prevention? *Gut* 2006, *55*, 1387–1389.

[10] Gnagnarella, P., Salvini, S., Parpinel, M., Food Composition Database for Epidemiological Studies in Italy, Version 1. Available from http://www.ieo.it/bda. 2008.

[11] Park, J. Y., Nicolas, G., Freisling, H., Biessy, C. et al., Comparison of standardised dietary folate intake across ten countries participating in the European Prospective Investigation into Cancer and Nutrition. *Br. J. Nutr.* 2012, *108*, 552–569.

[12] Winkels, R. M., Brouwer, I. A., Siebelink, E., Katan, M. B. et al., Bioavailability of food folates is 80% of that of folic acid. *Am. J. Clin. Nutr.* 2007, *85*, 465–473.

[13] Ulrich, C. M., Xu, X., Liu, A., Chen, J., Folate. In: Milner, J. A., Romagnolo, D. F., (Eds.), *Bioactive Compounds and Cancer*, 2010, Humana Press, Springer, New York, pp. 387–410.

[14] Wirfält, A. K. E., Jeffery, R. W., Elmer, P. J., Comparison of food frequency questionnaires: the reduced block and Willett

questionnaires differ in ranking on nutrient intakes. *Am. J. Epidemiol.* 1998, *148*, 1148–1156.

[15] Bingham, S. A., Luben, R., Welch, A., Wareham, N. et al., Are imprecise methods obscuring a relation between fat and breast cancer? *Lancet* 2003, *362*, 212–214.

[16] Henriquez-Sanchez, P., Sanchez-Villegas, A., Doreste-Alonso, J. et al., Dietary assessment methods for micronutrient intake: a systematic review on vitamins. *Br. J. Nutr.* 2009, *102* (Suppl 1), S10–S37.

[17] Bingham, S. A., Biomarkers in nutritional epidemiology. *Public Health Nutr*. 2002, *5*, 821–827.

[18] Gibson, R. S., *Principles of Nutritional Assessment*. Oxford University Press, New York 2005.

[19] Kipnis, V, Midthune, D., Freedman, L. S. et al., Empirical evidence of correlated biases in dietary assessment instruments and its implications. *Am. J. Epidemiol.* 2001, *153*, 394–403.

[20] Kaaks, R., Riboli, E., Sinha, R, Biochemical markers of dietary intake. In: *Application of Biomarkers in Cancer Epidemiology*, Vol. *142*, 1997, IARC Scientific Publications, Lyon, pp. 103–128.

[21] Jenab, M., Slimani, N., Bictash, M., Ferrari, P. et al., Biomarkers in nutritional epidemiology: applications, needs and new horizons. *Hum. Genet.* 2009, *125*, 507–525.

[22] Willett, W. C., *Nutritional Epidemiology*, 2nd edition. Oxford University Press, New York, 1998.

[23] Kipnis, V., Subar, A. F., Midthune, D. et al., The structure of dietary measurement error: results of the OPEN biomarker study. *Am. J. Epidemiol.* 2003, *158*, 14–21.

[24] Crews, H., Alink, G., Andersen, R. et al., A critical assessment of some biomarker approaches linked with dietary intake. *Br. J. Nutr.* 2001, *86*(Suppl 1), S5–S35.

[25] Flood, V. M., Smith, W. T., Webb, K. L., Mitchell, P., Issues in assessing the validity of nutrient data obtained from a food frequency questionnaire: folate and vitamin B12 examples. *Public Health Nutr.* 2004, *7*, 751–756.

[26] Bacardí-Gascón, M., de Góngoraa, S., Castro-Vβzquez, B. Y., Jiménez-Cruza, A., Validation of a semiquantitative food frequency questionnaire to assess folate status. Results discriminate a high-risk group of women residing on the Mexico-U.S. border. *Arch. Med. Res.* 2007, *34*, 325–330.

[27] Colic Baric, I., Satalic, Z., Keser, I., Cecic, I. et al., Validation of the folate food frequency questionnaire with serum and erythrocyte folate and plasma homocysteine. *Int. J. Food. Sci. Nutr.* 2009, *60*(Suppl 5), 10–18.

[28] Colic Baric, I., Satalic, Z., Pedisic, Z., Zizic, V. et al., Validation of the folate food frequency questionnaire in vegetarians. *Int. J. Food. Sci. Nutr.* 2009, *60*(Suppl 5), 88–95.

[29] Drogan, D., Klipstein-Grobusch, K., Wans, S., Luley, C. et al., Plasma folate as marker of folate status in epidemiological studies: the European investigation into cancer and nutrition (EPIC)-Potsdam study. *Br. J. Nutr.* 2004, *92*, 489–496.

[30] Fayet, F., Flood, V., Petocz, P., Samman, S., Relative and biomarker-based validity of a food frequency questionnaire that measures the intakes of vitamin B12, folate, iron, and zinc in young women. *Nutr. Res.* 2011, *31*, 14–20.

[31] Hickling, S., Knuiman, M., Jamrozik, K., Hung, J., A rapid dietary assessment tool to determine intake of folate was developed and validated. *J. Clin. Epidemiol.* 2005, *58*, 802–808.

[32] Iso, H., Moriyama, Y., Yoshino, K., Sasaki, S. et al., Validity of the self-administered food frequency questionnaire used in the 5-year follow-up survey for the JPHC Study to assess folate, vitamin B6 and B12 intake: comparison with dietary records and blood level. *J. Epidemiol.* 2003, *13*, S98–S101.

[33] Jackson, M., Walker, S., Younger, N., Bennett, F., Use of a food frequency questionnaire to assess diets of Jamaican adults: validation and correlation with biomarkers. *Nutr. J.* 2011, *10*, 28.

[34] Johansson, I., Van Guelpen, B., Hultdin, J., Johansson, M. et al., Validity of food frequency questionnaire estimated intakes of folate and other B vitamins in a region without folic acid fortification. *Eur. J. Clin. Nutr.* 2010, *64*, 905–913.

[35] Knutsen, S. F., Fraser, G. E., Linsted, K. D., Beeson, W. L. et al., Comparing biological measurements of vitamin C, folate, alpha-tocopherol and carotene with 24-hour dietary recall information in nonhispanic blacks and whites. *Ann. Epidemiol*. 2001, *11*, 406–416.

[36] Pufulete, M., Emery, P. W., Nelson, M., Sanders, T. A., Validation of a short food frequency questionnaire to assess folate intake. *Br. J. Nutr.* 2002, *87*, 383–390.

[37] Shai, I., Rosner, B. A., Shahar, D. R. et al., Dietary evaluation and attenuation of relative risk: multiple comparisons between blood and urinary biomarkers, food frequency, and 24-hour recall questionnaires: the DEARR study. *J. Nutr.* 2005, *135*, 573–579.

[38] Shuaibi, A. M., Sevenhuysen, G. P., House, J. D., Validation of a food choice map with a 3-day food record and serum values to assess folate and vitamin B12 intake in college-aged women. *J. Am. Diet. Assoc.* 2008, *108*, 2041–2050.

[39] Signorello, L. B., Buchowski, M. S., Cai, Q., Munro, H. M. et al., Biochemical validation of food frequency questionnaire-estimated carotenoid, alpha-tocopherol, and folate intakes among African Americans and non-Hispanic whites in the southern community cohort study. *Am. J. Epidemiol.* 2010, *171*, 488–497.

[40] van de Rest, O., Durga, J., Verhoef, P., Melse-Boonstra, A. et al., Validation of a food frequency questionnaire to assess folate intake of Dutch elderly people. *Br. J. Nutr.* 2007, *98*, 1014–1020.

[41] Verkleij-Hagoort, A. C., de Vries, J. H. M., Stegers, M. P. G., Lindemans, J. et al., Validation of the assessment of folate and vitamin B12 intake in women of reproductive age: the method of triads. *Eur. J. Clin. Nutr.* 2006, *61*, 610–615.

[42] Yen, J., Zoumas-Morse, C., Pakiz, B., Rock, C. L., Folate intake assessment: validation of a new approach. *J. Am. Diet. Assoc.* 2003, *103*, 991–1000.

[43] Ferlay, J., Parkin, D. M., Steliarova-Foucher, E., Estimates of cancer incidence and mortality in Europe in 2008. *Eur. J. Cancer* 2010, *46*, 765–781.

[44] Institute of Medicine, *National Academy of Sciences USA. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin,*

Vitamin B6, Folate, Vitamin B12, Pantothenic acid, Biotin and Choline. National Academies Press, Washington, DC 1998.

[45] Bailey, L. B., Dietary reference intakes for folate: the debut of dietary folate equivalents. *Nutr. Rev.* 1998, *56*, 294–299.

[46] Cade, J., Thompson, R., Burley, V., Warm, D., Development, validation and utilisation of food frequency questionnaires—a review. *Public Health Nutr.* 2002, *5*, 567–587.

[47] Bland, J. M., Altman, D. G., Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet* 1995, *346*, 1085–1087.

[48] Bland, J. M., Altman, D. G., Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986, *1*, 307–310.

[49] Schmidt, M. E., Steindorf, K., Statistical methods for the validation of questionnaires—discrepancy between theory and practice. *Methods Inf. Med.* 2006, *45*, 409–413.

[50] Cohen, J., A coefficient of agreement for nominal scales. *Educ. Psychol. Meas.* 1960, *20*, 37–46.

[51] Cohen, J., Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. *Psychol. Bull.* 1968, *70*, 213–220.

[52] Masson, L. F., MCNeill, G., Tomany, J. O. et al., Statistical approaches for assessing the relative validity of a food frequency questionnaire: use of correlation coefficients and the kappa statistic. *Public Health Nutr.* 2003, *6*, 313–321.

[53] Ocké, M. C., Kaaks, R. J., Biochemical markers as additional measurements in dietary validity studies: application of the method of triads with examples from the European prospective investigation into cancer and nutrition. *Am. J. Clin. Nutr.* 1997, *65*, 1240S–1245S.

[54] Fraser, G. E., Butler, T. L., Shavlik, D., Correlations between estimated and true dietary intakes: using two instrumental variables. *Ann. Epidemiol.* 2005, *15*, 509–518.

[55] Capita, R., onso-Calleja, C., Differences in reported winter and summer dietary intakes in young adults in Spain. *Int. J. Food. Sci. Nutr.* 2005, *56*, 431–443.

[56] Cox, B. D., Whichelow, M. J., Prevost, A. T., Seasonal consumption of salad vegetables and fresh fruit in relation to the development of cardiovascular disease and cancer. *Public Health Nutr.* 2000, *3*, 19–29.

[57] Melse-Boonstra, A., de Bree, A., Verhoef, P., Bjørke-Monsen, A. et al., Dietary monoglutamate and polyglutamate folate are associated with plasma folate concentrations in Dutch men and women aged 20–65 years. *J. Nutr.* 2002, *132*, 1307–1312.

[58] Krebs-Smith, S. M., Heimendinger, J., Subar, A. F., Patterson, B. H. et al., Using food frequency questionnaires to estimate fruit and vegetable intake: association between the number of questions and total intakes. *J. Nutr. Educ.* 1995, *27*, 80–85 (Abstract).

[59] Bouckaert, K. P., Slimani, N., Nicolas, G., Vignat, J. et al., Critical evaluation of folate data in European and international databases: recommendations for standardization in international nutritional studies. *Mol. Nutr. Food. Res.* 2011, *55*, 166–180.

[60] Deharveng, G., Charrondiere, U. R., Slimani, N., Southgate, D. A. et al., Comparison of nutrients in the food composition tables available in the nine European countries participating in EPIC. European prospective investigation into cancer and nutrition. *Eur. J. Clin. Nutr.* 1999, *53*, 60–79.

[61] Block, G., Sinha, R., Gridley, G., Collection of dietary-supplement data and implications for analysis. *Am. J. Clin. Nutr.* 1994, *59*, 232S–239S.

[62] Messerer, M., Johansson, S. E., Wolk, A., The validity of questionnaire-based micronutrient intake estimates is increased by including dietary supplement use in swedish men. *J. Nutr.* 2004, *134*, 1800–1805.

[63] Sieri, S., Krogh, V, Saieva, C. et al., Alcohol consumption patterns, diet and body weight in 10 European countries. *Eur. J. Clin. Nutr.* 2009, *63*, S81–S100.

[64] Marshall, J. R., Methodologic and statistical considerations regarding use of biomarkers of nutritional exposure in epidemiology. *J. Nutr.* 2003, *133*, 881S–887S.

[65] Brouwer, I. A., van Dusseldorp, M., West, C. E. et al., Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. *J. Nutr.* 1999, *129*, 1135–1139.

[66] Appel, L. J., Miller, E. R., Jee, S. H. et al., Effect of dietary patterns on serum homocysteine : results of a randomized, controlled feeding study. *Circulation* 2000, *102*, 852–857.

[67] Kondo, A., Asada, Y., Shibata, K. et al., Dietary folate intakes and effects of folic acid supplementation on folate concentrations among Japanese pregnant women. *J. Obstet. Gynaecol. Res.* 2011, *37*, 331–336.

[68] McNulty, H., Cuskelly, G. J., Ward, M., Response of red blood cell folate to intervention: implications for folate recommendations for the prevention of neural tube defects. *Am. J. Clin. Nutr.* 2000, *71*, 1308S–1311S.

[69] Houghton, L. A., Gray, A. R., Rose, M. C., Miller, J. C., Hurthouse, N. A., Gregory, J. F., Long-term effect of low-dose folic acid intake: potential effect of mandatory fortification on the prevention of neural tube defects. *Am. J. Clin. Nutr.* 2011, *94*, 136–141.

[70] Venn, B. J., Mann, J. I., Williams, S. M. et al., Dietary counseling to increase natural folate intake: a randomized, placebo-controlled trial in free-living subjects to assess effects on serum folate and plasma total homocysteine. *Am. J. Clin. Nutr.* 2002, *76*, 758–765.

[71] van Oort, F. V., Melse-Boonstra, A., Brouwer, I. A. et al., Folic acid and reduction of plasma homocysteine concentrations in older adults: a dose-response study. *Am. J. Clin. Nutr.* 2003, *77*, 1318–1323.

[72] Schatzkin, A., Subar, A. F., Moore, S. et al., Observational epidemiologic studies of nutrition and cancer: the next generation (with better observation). *Cancer Epidemiol. Biomarkers Prev.* 2009, *18*, 1026–1032.

[73] Sanderson, P., McNulty, H., Mastroiacovo, P. et al., Folate bioavailability: UK Food Standards Agency workshop report. *Br. J. Nutr.* 2003, *90*, 473–479.

[74] Bohnsack, B. L., Hirschi, K. K., Nutrient regulation of cell cycle progression. *Annu. Rev. Nutr.* 2004, *24*, 433–453.

[75] Green, T. J., Allen, O. B., O'Connor, D. L., A three-day weighed food record and a semiquantitative food – frequency questionnaire are valid measures for assessing the folate and vitamin B12 intakes of women aged 16 to 19 years. *J. Nutr.* 1998, *128*, 1665–1671.

[76] Owen, W. E., Roberts, W. L., Comparison of five automated serum and whole blood folate assays. *Am. J. Clin. Pathol.* 2003, *120*, 121–126.

[77] Wright, A. J. A., Finglas, P. M., Southon, S., Erythrocyte folate analysis: a cause for concern? *Clin. Chem.* 1998, *44*, 1886–1891.

[78] Pfeiffer, C. M., Gregory, J. F., Enzymatic deconjugation of erythrocyte polyglutamyl folates during preparation for folate assay: investigation with reversed-phase liquid chromatography. *Clin. Chem.* 1996, *42*, 1847–1854.

[79] Gunter, E. W., Bowman, B. A., Caudill, S. P., Twite, D. B. et al., Results of an international round robin for serum and whole-blood folate. *Clin. Chem.* 1996, *42*, 1689–1694.

[80] Hannisdal, R., Gislefoss, R. E., Grimsrud, T. K., Hustad, S. et al., Analytical recovery of folate and its degradation products in human serum stored at −25°C for up to 29 Years. *J. Nutr.* 2010, *140*, 522–526.

[81] Hannisdal, R., Ueland, P. M., Eussen, S. J. P. M., Svardal, A. R. et al., Analytical recovery of folate degradation products formed in human serum and plasma at room temperature. *J. Nutr.* 2009, *139*, 1415–1418.

[82] Jacques, P. F., Sulsky, S. I., Sadowski, J. A., Phillips, J. C. et al., Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. *Am. J. Clin. Nutr.* 1993, *57*, 182–189.

[83] Melse-Boonstra, A., Verhoef, P., West, C., Quantifying folate bioavailability: a critical appraisal of methods. *Curr. Opin. Clin. Nutr. Metab. Care* 2004, *7*, 539–545.

[84] Kaaks, R. J., Biochemical markers as additional measurements in studies of the accuracy of dietary questionnaire measurements: conceptual issues. *Am. J. Clin. Nutr.* 1997, *65*, 1232S–1239S.

[85] Yokota, R. T., Miyazaki, E. S., Ito, M. K., Applying the triads method in the validation of dietary intake using biomarkers. *Cad. Saude Publica* 2010, *26*, 2027–2037.

[86] Mander, A., *BATPLOT: Stata Module to Produce Bland–Altman Plots Accounting for Trend. (Statistical Software Components S448703)*. Boston College Department of Economics, Boston, 2005.

[87] Freedman, L, Kipnis, V, Schatzkin, A, Tasevska, N et al., Can we use biomarkers in combination with self-reports to strengthen the analysis of nutritional epidemiologic studies? *Epidemiol. Perspect. Innov.* 2010, *7*, 2.

