Systematic Review

Annals of Nutrition & Metabolism

Ann Nutr Metab 2018;73:30–43
DOI: 10.1159/000490003

Received: February 1, 2018
Accepted after revision: May 14, 2018
Published online: June 7, 2018

Systematic Review of Observational Studies with Dose-Response Meta-Analysis between Folate Intake and Status Biomarkers in Adults and the Elderly

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Keywords
Folate · Intake-status · Dose-response · Adults-elderly

Abstract
Background: Dietary reference values for folate intake vary widely across Europe. ***Methods:*** MEDLINE and Embase through November 2016 were searched for data on the association between folate intake and biomarkers (serum/plasma folate, red blood cell [RBC] folate, plasma homocysteine) from observational studies in healthy adults and elderly. The regression coefficient of biomarkers on intake (β) was extracted from each study, and the overall and stratified pooled β and SE (β) were obtained by random effects meta-analysis on a double log scale. These dose-response estimates may be used to derive folate intake reference values. ***Results:*** For every doubling in folate intake, the changes in serum/plasma folate, RBC folate and plasma homocysteine were +22, +21, and −16% respectively. The overall pooled regression coefficients were β = 0.29 (95% CI 0.21–0.37) for serum/plasma folate (26 estimates from 17 studies), β = 0.28 (95% CI 0.21–0.36) for RBC (13 estimates from 11 studies), and β = −0.21 (95% CI −0.31 to −0.11) for plasma homocysteine (10 estimates from 6 studies). ***Conclusion:*** These estimates along with those from randomized controlled trials can be used for underpinning dietary recommendations for folate in adults and elderly.

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Introduction

Adequate folate intake is necessary to achieve and maintain optimal health in all life stages. Folate deficiencies can result in many health problems, such as neural tube defects in developing embryos, and may lead to an increased risk of cardiovascular disease, anemia, cognitive impairment, and depression among adults and elderly [1–3]. Dietary reference values provide guidance on optimal dietary folate intake that should prevent deficiency and development of health problems.

The EURopean micronutrient RECommendations Aligned network (EURRECA; http://www. eurreca.org) has been developing approaches to derive and update micronutrient reference values, including folate. This process requires reliable data on the association between folate intake, status and health outcomes, which would allow estimating the intakes to achieve preset concentrations of relevant biomarkers. The EURRECA network has proposed 3 biomarkers of folate status: plasma or serum folate, red blood cell (RBC) folate, and plasma homocysteine (tHcy) [4]. A meta-analysis on dose-response relation between folic acid intake and plasma biomarkers from randomized controlled trials (RCTs) in adults and elderly has been recently investigated by EURRECA coworkers [3]. We now performed a systematic review that included data from observational studies in which folate intake represents intakes from its natural dietary matrix and from fortified foods and supplements, and in which populations are not confined to those strictly selected by the RCTs inclusion and exclusion criteria [5, 6]. This study fills a knowledge gap by systematically reviewing all available observational studies that investigated the relationship between folate intake and biomarkers of folate status in adults and elderly, followed by meta-analyses in order to model biomarkers of folate status as a function of dietary folate intake. Table 1 summarizes criteria used in the present systematic review.

Methods

Search Strategy

Within the EURRECA framework, systematic reviews were performed to explore the associations between intakes, status, and selected health outcomes for the public health priority micronutrients (iron, zinc, folate, vitamin B12, and iodine) [7–9]. This process followed a harmonized search strategy, aimed to collect the data published up to and including November 2016 using Medline and Embase search terms for ("study designs in humans") AND (folate or folic acid or vitamin B9) AND (intake OR status), retrieved on Ovid Platform. Both indexing and text terms were used and languages included were restricted to those spoken in the EURRECA Network (English, Dutch, French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek, and Serbian). The summary of the general search strategy developed for a search in EMBASE (on Ovid Platform) is shown in Table 2. Search strategies for MEDLINE (on Ovid Platform) were adapted based on this strategy. The reference lists of retrieved articles and published reviews were also checked for relevant studies. Where necessary, authors were contacted to provide missing data or clarify methods or results.

Study Selection

For a study to be included, it had to meet all of the following inclusion criteria: designed as prospective cohort, nested case-control or cross-sectional study in apparently healthy adults and elderly; it had to report on both dietary folate intake and status following the EURRECA guidance on best practice micronutrient intake and status method [9]; folate intake was measured with a validated food frequency questionnaire (FFQ), a dietary history, or a 24-h recall (24HDR) or a food record/diary (FR); folate status was reported as plasma or serum folate, RBC folate, or total plasma homocysteine a non-specific marker of folate status.

Studies were excluded if they were retrospective cohort studies, or non-nested case-control studies, performed in non-healthy or populations other than adults and elderly, or were commentaries, reviews, or duplicate publications from the same study.

Of 7,249 identified articles in the wider search on folate intake, status and priority health outcomes in all populations, 7,049 were excluded following the screening of the title and abstract. The remaining 200 full-text articles were screened by 2 independent reviewers (R.N. and M.N.) and all discrepancies on study inclusion were discussed. This process resulted in 45 potentially relevant papers, out of which 23 were identified for the final analysis, in addition to 1 paper that was identified by following up on reference lists of the articles that were included into this review. Papers with incomplete data that could not be obtained from the authors were excluded. The flow diagram of the articles screened, assessed, and excluded at various stages is shown in Figure 1.

Data Extraction

For each of the identified manuscripts, data were extracted into a standardized database. This was done by one (R.N.) and checked by another reviewer (M.N.).

Extracted data included population characteristics, mean, and SD of folate intake and dietary assessment method, concentrations of folate related biomarkers and analytical method used to measure folate status, the association and type of association between folate intake and folate-related biomarkers (Spearman rank correlation coefficient, Pearson correlation coefficient, linear regression coefficient), and information on any transformations applied to obtain the reported associations. Serum/plasma folate concentrations were converted to μmol/L when applicable.

In order to assess the quality of the included studies and the risk of bias, indicators of internal validity were collected during data extraction. Based on the indicators, 2 independent reviewers assessed the overall risk of bias and disagreements resolved by discussion. The criteria for judging these indicators were adapted from the Cochrane Library [10]. Table 3 presents the characteristics of the included studies [11–34].

Table 1. PICOS criteria used to define the research question for the systematic review

Criteria	Description
Population	Apparently healthy adults and elderly
Intervention	Folate intake from diet and supplements
Comparison group	The relationship between dietary folate intake and biomarkers of folate status: serum/plasma folate, red blood cell folate and/or plasma homocysteine
Outcomes	The overall and stratified regression coefficient of biomarkers of status on dietary folate intake
Study design	Observational studies

Table 2. Summary of the EURRECA general search strategy including the search terms specific for folate, developed for a search in EMBASE (Ovid)

Category	Search criteria
#1 Study design in humans	– Cohorts – Systematic reviews – Cross-sectional
#2 Intake OR status	– Supplementation/or diet supplementation/or dietary intake/or exp diet restriction/or exp mineral intake/or infant nutrition/or artificial milk/or breast milk/or bottle feeding/or breast feeding/or lactation/OR – Exp nutritional status/or nutritional deficiency/or exp folate deficiency/or exp folate blood level/OR – (Intake* or diet* or supplement* or deplet* or status or concentration* or expos* or fortif* or plasma or serum or "red blood cell*" or red blood cell or RBC or RCF or plasma homocysteine or hcy).ti, ab.
#3 Micronutrient	Folate or folic acid or vitamin B9
#1 AND #2 AND #3	

Data Synthesis

Each study provided at least one independent estimate; for some studies, 2 or 3 estimates were obtained because of stratification by gender [20, 25], by genotype [15, 17], by race [29, 32], or by diet type [23]. Where data on folate intake and status were available for males and females separately, but there was only one estimate of the association [13, 16, 25, 27]; the pooled estimates of mean folate intake and status were calculated and used in the analysis.

Statistical Analysis

The intake-status relationship was assumed to be linear with a coefficient between –1 and +1 on the $\log_e$–$\log_e$ scale (natural logarithm of dependent and independent variable). Positive values would translate to a monotonic concave curve on the arithmetic scale that steeply increases at low intakes and less so at higher levels, which is a common shape in biology [6, 35].

Assuming this model, summary statistics from each study were transformed into estimates of the dose-response relationship: a regression coefficient (β) and its standard error (SE [β]) of micronutrient status on micronutrient intake. The individual estimates of the dose-response regression coefficients were combined into an overall pooled β and SE (β) by means of random effects meta-analysis, which incorporates the between-study variation using the method of DerSimonian and Laird [36] to obtain the weights required for the summary estimate. Residual heterogeneity between studies was evaluated using the I^2 statistic. We investigated the intake-status relationship for each of the 3 biomarkers separately. In addition, we investigated whether mean age (continuous), dietary intake method (categories: [i] FFQ, and [ii] 24HDR or FR), and folate matrix (categories: [i] folate from the diet, [ii] folate from the diet and supplements), and analytical method used to measure folate status (categories: [i] microbiological assay, [ii] non microbiological assay) were variables that modified the association using meta-regression. Statistical transformations to obtain β and SE (β) were performed using GenStat version 13-SP2 (VSN International Ltd., http://www.vsni.co.uk/) and the meta-analysis was performed using STATA version 10.0 (College Station, TX, USA), with statistical significance defined as $p < 0.05$.

All studies that were included in this review were assessed for study quality by following the Cochrane Handbook. It was checked if these studies dealt with confounding factors adequately, whether assessment of exposure (intake or status) and funder were ade-

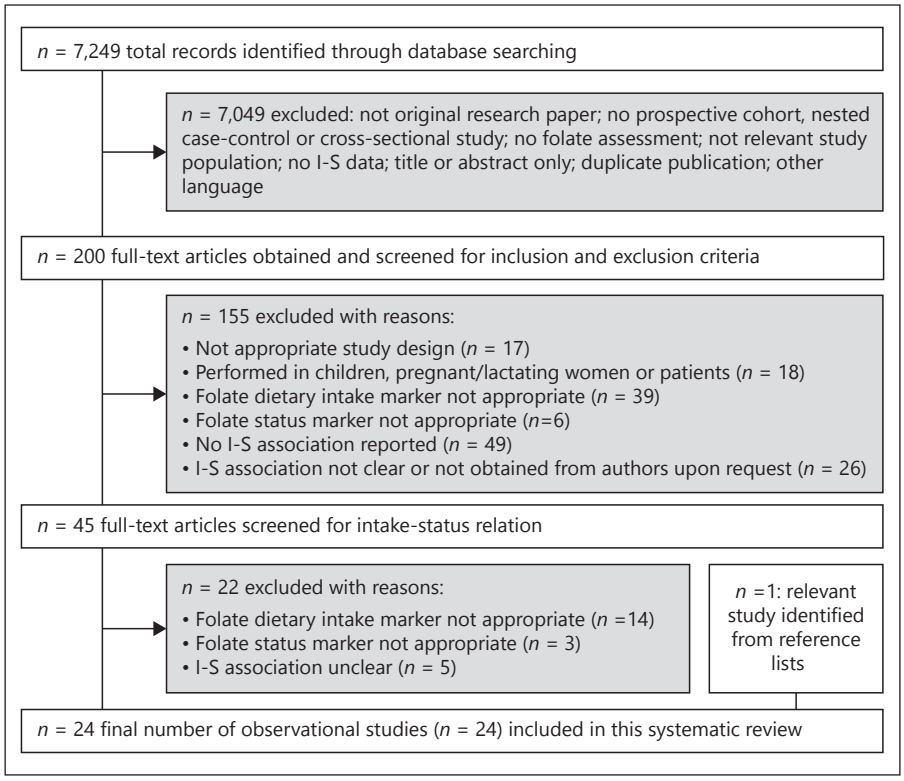

Fig. 1. Flow diagram of the study selection process for the dose-response meta-analysis between folate intake and status biomarkers in adults and elderly. I, intake; S, status, referring to a biomarker of intake.

quate, and if there were other potential threats to validity (online suppl. Table 1, see www.karger.com/doi/10.1159/000490003). Every study was assigned judgments such as "high," "moderate," or "low" risk for the purpose of study quality categorization. A consensus on the judgments was reached following discussion of each study. Thirteen studies [12, 15, 17, 19–25, 27, 30, 31] were judged at adequate risk of bias, 6 studies [14, 16, 18, 26, 29, 32, 34] were judged at a moderate risk of bias, and 4 studies was judged at a high risk of bias [11, 13, 28, 33].

Results

Serum/Plasma Folate

We identified 26 estimates from 17 observational studies of folate intake and serum/plasma folate status that were eligible for our meta-analysis. Two papers reported results separately for 3 different genotypes, 1 paper reported results separately for 3 diet types, and 3 papers reported results for males and females separately. In total, 8,344 subjects were included with mean ages ranging from 21 to 65 years and sample sizes that ranged from 53 to 1,275 participants (Table 3). Most associations were obtained from cross-sectional analyses ($n = 18$), although some were derived from nested case-control studies ($n = 6$) and from a prospective cohort study ($n = 1$).

Combining these estimates in one meta-analysis yielded an overall pooled beta-coefficient of $\beta = 0.29$ (95% CI 0.21–0.37; $I^2 = 92.2\%$; Fig. 2) [11–27]. So, for studies in the range 152–662 μg/day, doubling of a folate intake resulted in an increase in serum/plasma folate by 2^{β} or 22% ($2^{0.29} = 1.22$). Thus, for example, an average person with a folate intake of 100 μg/day has a serum/plasma folate status concentration that is 22% higher than a person who has a folate intake of 50 μg/day, or an average person with a folate intake of 200 μg/day has a serum/plasma folate status concentration that is 22% higher than a person who has a folate intake of 100 μg/day.

As the estimates showed substantial heterogeneity between the studies, the meta-analysis was subsequently stratified for folate matrix and dietary intake method. Stratification according to the matrix of provided folate (categories: [i] folate from the diet, [ii] folate from the diet and supplements, whether or not converted to dietary folate equivalents) yielded significantly different estimates of $\beta = 0.33$ (95% CI 0.22–0.44; $n = 16$) for dietary folate and $\beta = 0.22$ (95% CI 0.12–0.33; $n = 10$) for dietary folate plus supplements (Fig. 2). This difference was not statistically significant ($p = 0.20$) and the heterogeneity within the strata remained high ($I^2 = 92.3$ and 86.6%).

Table 3. General characteristics of the included observational studies in adults and elderly reporting the association between folate intake and the biomarkers of folate status: plasma or serum folate, red blood cell folate, and plasma homocysteine

First author, year	*n*	Gender	Age, years, mean	Folate intake, μg/day	Source of folate intake[1]	Dietary assessment method	Analytical method
Plasma or serum folate							
Arnaud et al. [11], 2001	106	M	39	152.0	Diet	Food record (7 days)	Protein-binding assay
Ashfield-Watt et al. [12], 2003	133	M&F	41	242.0	Diet	FFQ	Protein-binding assay
Chew et al. [13], 2010	100	M&F	28	296.0	Diet	1 × 24HDR	Microbiological assay*
Colic Baric et al. [14], 2009	99	F	53	252.6	Diet and supplements[1]	FFQ	Ion-capture-assay
de Bree et al. [15], 2003, CC genotype	938	M&F	41	205.0	Diet	FFQ	Lact. caseimicrob. assay*
de Bree et al. [15], 2003, CT genotype	907	M&F	41	203.0	Diet	FFQ	Lact. caseimicrob. assay*
de Bree et al. [15], 2003, TT genotype	206	M&F	41	201.0	Diet	FFQ	Lact. caseimicrob. assay*
Drogan et al. [16], 2004	363	M&F	52	226.9	Diet and supplements[1]	FFQ	Ion-capture-assay
Fukuda et al. [17], 2004, CC genotype	199	M&F	65	308.0	Diet	FFQ	Auto-analyzer
Fukuda et al. [17], 2004, CT genotype	274	M&F	65	307.0	Diet	FFQ	Auto-analyzer
Fukuda et al. [17], 2004, TT genotype	81	M&F	62	299.0	Diet	FFQ	Auto-analyzer
Glyn et al. [18], 1996	249	M	60	320.0	Diet and supplements	FFQ	Radioassay
Johansson et al. [19], 2010, males	96	M	45	196.0	Diet and supplements	10 × 24HDR	Radioassay
Johansson et al. [19], 2010, females	99	F	45	181.0	Diet and supplements	10 × 24HDR	Radioassay
Melse-Boonstra et al. [20], 2002, males	1,275	M	41	232.0	Diet	FFQ	Microbiological assay*
Melse-Boonstra et al. [20], 2002, females	1,160	F	41	186.0	Diet	FFQ	Microbiological assay*
Sedjo et al. [21], 2002	179	F	27	418.6	Diet and supplements	FFQ	Radioassay
Shuaibi et al. [22], 2008	70	F	21	447.9	Diet and supplements[1]	Food record (3 days)	Radioassay
Shupbach et al. [23], 2015, Omn	100	M&F	32	281.0	Diet	Food record (3 days)	Microbiological assay*
Shupbach et al. [23], 2015, Vgn	53	M&F	30	662.0	Diet and supplements	Food record (3 days)	Microbiological assay*
Shupbach et al. [23], 2015, Vgt	53	M&F	31	368.0	Diet	Food record (3 days)	Microbiological assay*
Sivaprasad et al. [24], 2016	276	M&F	47	166.0	Diet and supplements	3 × 24HDR	Radioassay
Van Guelpen et al. [25], 2009, males	704	M	51	249.0	Diet and supplements	FFQ	Radioassay
Van Guelpen et al. [25], 2009, females	293	F	60	249.0	Diet and supplements	FFQ	Radioassay
Verkleij-Hagoort et al. [26], 2007	53	F	32	177.0	Diet	FFQ	EDTA Hemat. Anal.
Weinstein et al. [27], 2008	278	M	58	330.7	Diet	FFQ	Radioassay
Red blood cell folate							
Chew et al. [13], 2010	100	M&F	28	296.0	Diet	1 × 24HDR	Microbiological assay*
Colic Baric et al. [14], 2009	99	F	52	252.6	Diet and supplements[1]	FFQ	Ion-capture-assay
Drogan et al. [16], 2004	363	M&F	52	226.9	Diet and supplements[1]	FFQ	Ion-capture-assay
Fayet et al. [28], 2010	53	F	22	383.0	Diet	3 × 24HDR	Chemiluminescent assays
Fraser et al. [29], 2016, B	373	M&F	56	469.0	Diet and supplements	6 × 24HDR	Chemiluminescent assays
Fraser et al. [29], 2016, NB	462	M&F	61	592.0	Diet and supplements	6 × 24HDR	Chemiluminescent assays
Hoey et al. [30], 2007	439	M&F	62	250.3	Diet	Food diary (4 days)	Microbiological assay*

Table 3. (continued)

First author, year	*n*	Gender	Age, years, mean	Folate intake, μg/day	Source of folate intake[1]	Dietary assessment method	Analytical method
Hopkins et al. [31], 2015	760	M&F	47	343.2	Diet and supplements[1]	Food diary (4 days)	Microbiological assay*
Knutsen et al. [32], 2001, NHB	97	M&F	47	306.6	Diet	8 × 24HDR	Radioimunoassay
Knutsen et al. [32], 2001, NHW	96	M&F	53	372.7	Diet	8 × 24HDR	Radioimunoassay
Mendoca et al. [33], 2016	732	M&F	86	209.0	Diet	2 × 24HDR	Chemiluminescent assays
Owens et al. [34], 2007	370	M&F	44	524.0	Diet and supplements[1]	FFQ	Chemiluminescent assays
Verkleij-Hagoort et al. [26], 2007	53	F	32	177.0	Diet	FFQ	EDTA Hemat. Anal.
Plasma homocysteine							
Chew et al. [13], 2010	100	M&F	28	296.0	Diet	1 × 24HDR	HPLC
Colic Baric et al. [14], 2009	99	F	53	252.6	Diet and supplements[1]	FFQ	Fluor. Polar. Imm.
de Bree et al. [15], 2003, CC genotype	938	M&F	41	205.0	Diet	FFQ	Protein-bound assay
de Bree et al. [15], 2003, CT genotype	907	M&F	41	203.0	Diet	FFQ	Protein-bound assay
de Bree et al. [15], 2003, TT genotype	206	M&F	41	201.0	Diet	FFQ	Protein-bound assay
Fukuda et al. [17], 2004, CC genotype	199	M&F	65	308	Diet	FFQ	Auto-analyzer
Fukuda et al. [17], 2004, CT genotype	274	M&F	65	307	Diet	FFQ	Auto-analyzer
Fukuda et al. [17], 2004, TT genotype	81	M&F	62	299	Diet	FFQ	Auto-analyzer
Van Guelpen et al. [25], 2009	293	F	60	249.0	Diet and supplements	FFQ	Immunoassay
Weinstein et al. [27], 2008	278	M	58	330.7	Diet	FFQ	HPLC

[1] Study that reported total folate intake as folate from diet and supplements converted into dietary folate equivalents DFE/day, other studies reported folate in μg/day.
* Microbiological assays.
n, number of subjects; M, males; F, females; 24HDR, 24 h diet recall; FFQ, food frequency questionnaire; Omn, omnivores; Vgt, vegetarians; Vgn, vegans; B, black; NB, non-black; NHB, nonhispanic blacks; NHW, nonhispanic whites; Lact. casei microb. assay, lactobacillus casei microbiological assay; EDTA Hemat. Anal., ethylene-diamine-tetra-acetate hematological analyses; Fluor. Polar. Imm., fluorescence polarization immunoassay; HPLC, high pressure liquid chromatography.

Stratification for dietary intake method yielded different estimates for FFQs (β = 0.33, 95% CI 0.22–0.43; n = 17) and 24HDR or FR (β = 0.18, 95% CI 0.10–0.25; n = 9), although this may be attributed to chance, as the difference was not statistically significant (p = 0.19). The between-study heterogeneity (I^2) remained high in the FFQ subgroup: it was 93.8% and moderate within 24HDR and FR – it was 38.7%.

Stratification for the analytical method used to measure serum/plasma folate (categories: [i] microbiological assay, [ii] non microbiological assay) yielded statistically different estimates of β = 0.51 (95% CI 0.37–0.64; n = 9) for microbiological assay and β = 0.18 (95% CI 0.14–0.22; n = 17) for non-microbiological assay (p < 0.0001). Studies that assessed serum/plasma folate response using the microbiological assay yielded somewhat higher heterogeneity (I^2 = 87.3%) compared to studies that assessed folate response using non-microbiological assays (I^2 = 49.2%).

The mean age of the study participants (continuous variable) was not a statistically significant determinant of the overall association (p = 0.52).

RBC Folate

We identified 11 observational studies of folate intake and RBC folate status that were eligible for our meta-analyses of which 4 also provided data on serum/plasma folate status. As 2 papers reported results by race separately, a total of 13 estimates were available for RBC folate, including a total of 3,997 subjects with mean age from 22 to 86 years, and sample sizes ranging from 53 to 760 subjects (Table 3). All studies were cross-sectional except 2 that dealt with prospective cohorts [32, 33], from which cross-sectional baseline data were used. Combining the 13 estimates in one meta-analysis yielded an overall pooled beta-coefficient β = 0.28 (95% CI 0.21–0.36; I^2 = 88.1%; Fig. 3) [13, 14, 16, 26, 28–34]. So, for studies in the range from 177 to 592 μg/day for every doubling in folate intake, the increase in RBC fo-

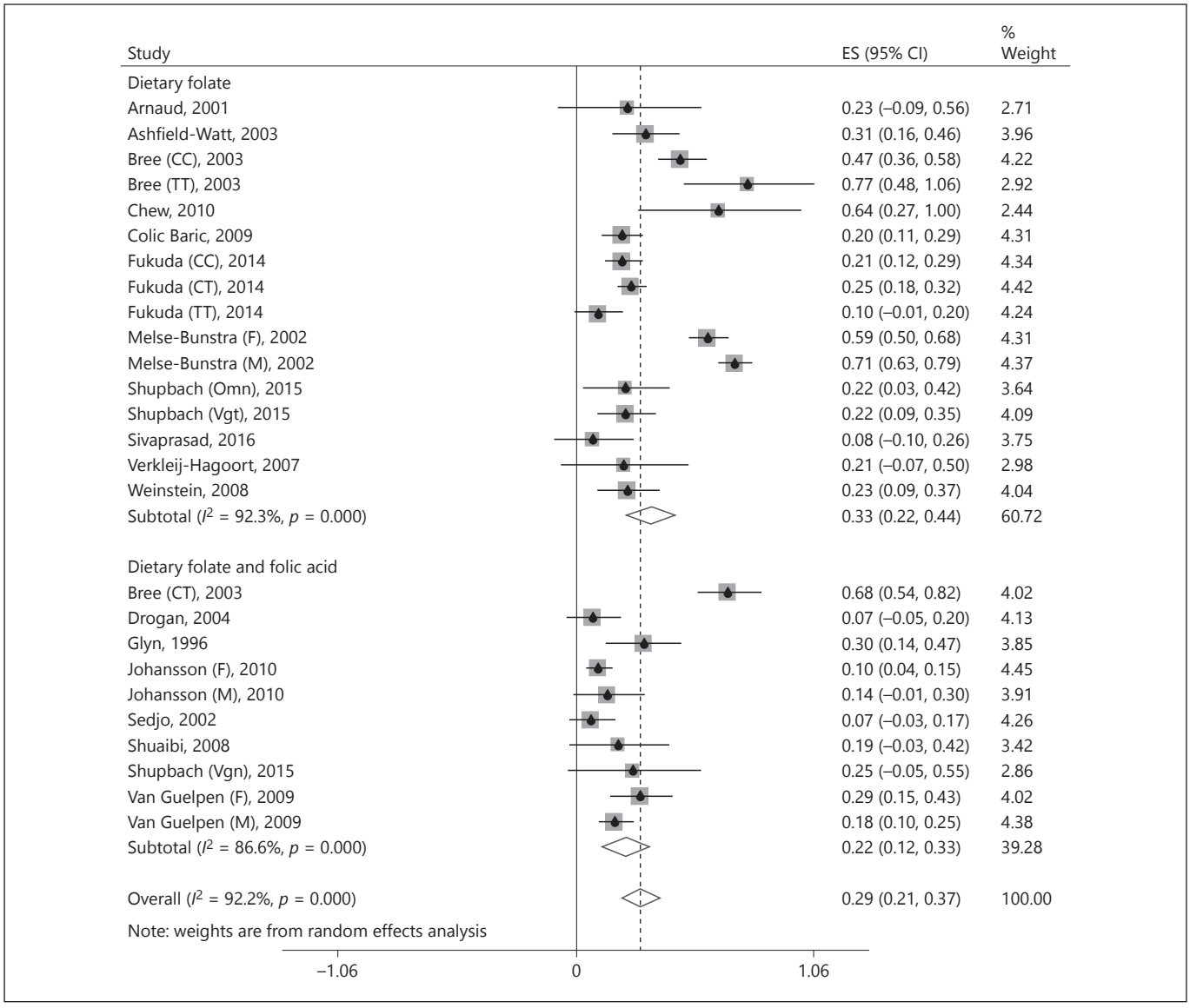

Fig. 2. Random effects meta-analysis of the association between dietary folate and serum/plasma folate in adults and elderly, stratified for the matrix of provided folate. The regression coefficients (β) represent the linear association between $\log_e$ transformed folate intake and $\log_e$ transformed serum/plasma folate status (lines represent the confidence intervals of each study). "Dietary folate" shows natural food folate expressed in μg/day in all studies; "Dietary folate including folic acid" shows natural food folate plus folic acid from supplements and fortified foods expressed in μg/day in all studies, except 3 in which it was expressed in DFE [14, 16, 22] (Table 3).

late concentration is 2^{β} or 21% ($2^{0.28} = 1.21$). Stratifying the analysis for the matrix of provided folate (categories: [i] folate from the diet, [ii] folate from the diet and supplements) yielded different estimates (β = 0.31, 95% CI 0.17–0.45, n = 6 and β = 0.27, 95% CI 0.17–0.36, n = 7 respectively), although they did not differ significantly and heterogeneity remained high (I^2 = 75.7 and 92.3%, respectively; Fig. 3). Stratifying the analysis for the dietary intake method (categories: [i] FFQ and [ii] 24HDR or FR) yielded estimates of β = 0.16, 95% CI 0.12–0.19 (n = 4 estimates) for FFQ, and β = 0.33, 95% CI 0.25–0.41 (n = 9 estimates) for 24HDR or FR, and reduced the between-study heterogeneity in the FFQ subgroup, but not in the latter subgroup (I^2 = 0 and 82.3% respectively). Stratification for analytical method used to measure RBC folate yielded similar estimates of β = 0.30 (95% CI 0.11–0.49 ; n = 3) for microbiological assay and

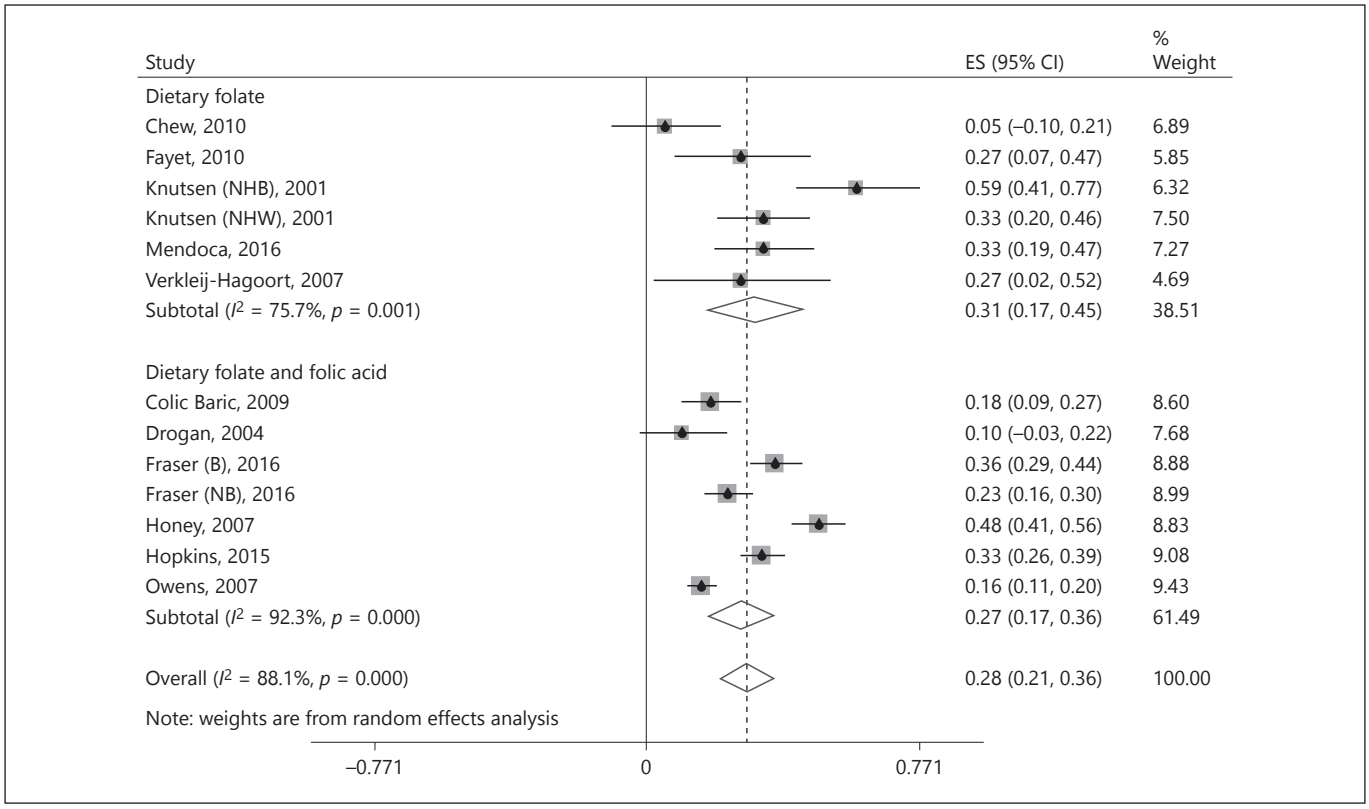

Fig. 3. Random effects meta-analysis of the association between dietary folate and red blood cell (RBC) folate in adults and elderly, stratified for the matrix of provided folate. The regression coefficients (β) represent the linear association between $\log_e$ transformed folate intake and $\log_e$ transformed RBC folate status (lines represent the confidence intervals of each study). "Dietary folate" shows natural food folate expressed in μg/day in all studies; "Dietary folate including folic acid" shows natural food folate plus folic acid from supplements and fortified foods expressed in μg/day in one study [26], whereas in others it was expressed in DFE [14, 16, 34] (Table 3).

β = 0.27 (95% CI 0.19–0.35; n = 10) for non-microbiological assay (p = 0.77). This subgroup analyses did not substantially reduce heterogeneity: I^2 = 92.4 and 82.1% for microbiological and non-microbiological assay respectively. Each of these potential sources of heterogeneity (dietary matrix, dietary intake method, and analytical method used to measure RBC folate) was evaluated continuously and simultaneously in a meta-regression model. The results showed that none of the potential sources of heterogeneity, namely, the dietary intake method (p = 0.20), the matrix of provided folate (p = 0.95), analytical method used to measure RBC folate (p = 0.87), and the mean age of the study participants (continuous variable; p = 0.51) were statistically significant determinants of the overall beta.

Plasma Homocysteine

We identified 6 observational studies of folate intake and plasma homocysteine status that were eligible for our meta-analyses; these studies also gave information on serum/plasma folate status. Two papers reported results separately for 3 different genotypes. In total, we had 10 estimates for plasma homocysteine including a total of 3,375 subjects with mean age from 28–65 years, and with sample sizes ranging from 81 to 938 subjects (Table 3). All studies were cross-sectional. Combining the 6 observational studies yielded an overall pooled beta of β = –0.21 (95% CI –0.31 to β = –0.11; I^2 = 90.5%; Fig. 4) [13–15, 17, 25, 27]. Thus, for studies in the range from 201 to 331 μg/day, a doubling of folate intake goes together with a difference in plasma homocysteine concentration of 2^{β} ($2^{-0.26}$ = 0.84), which is –16%. Stratifying the analysis for the matrix of provided folate (categories: [i] folate from the diet, [ii] folate from the diet and supplements) yielded different estimates (β = –0.23, 95% CI –0.37 to –0.10 [n = 8 estimates] and –0.13, 95% CI –0.18 to –0.07 [n = 2 estimates] respectively Fig. 4). They did not differ significantly (p = 0.58) and the heterogeneity was reduced only in the latter subgroup (I^2 = 0%), whereas in the former, it remained high (I^2 = 91.7%).

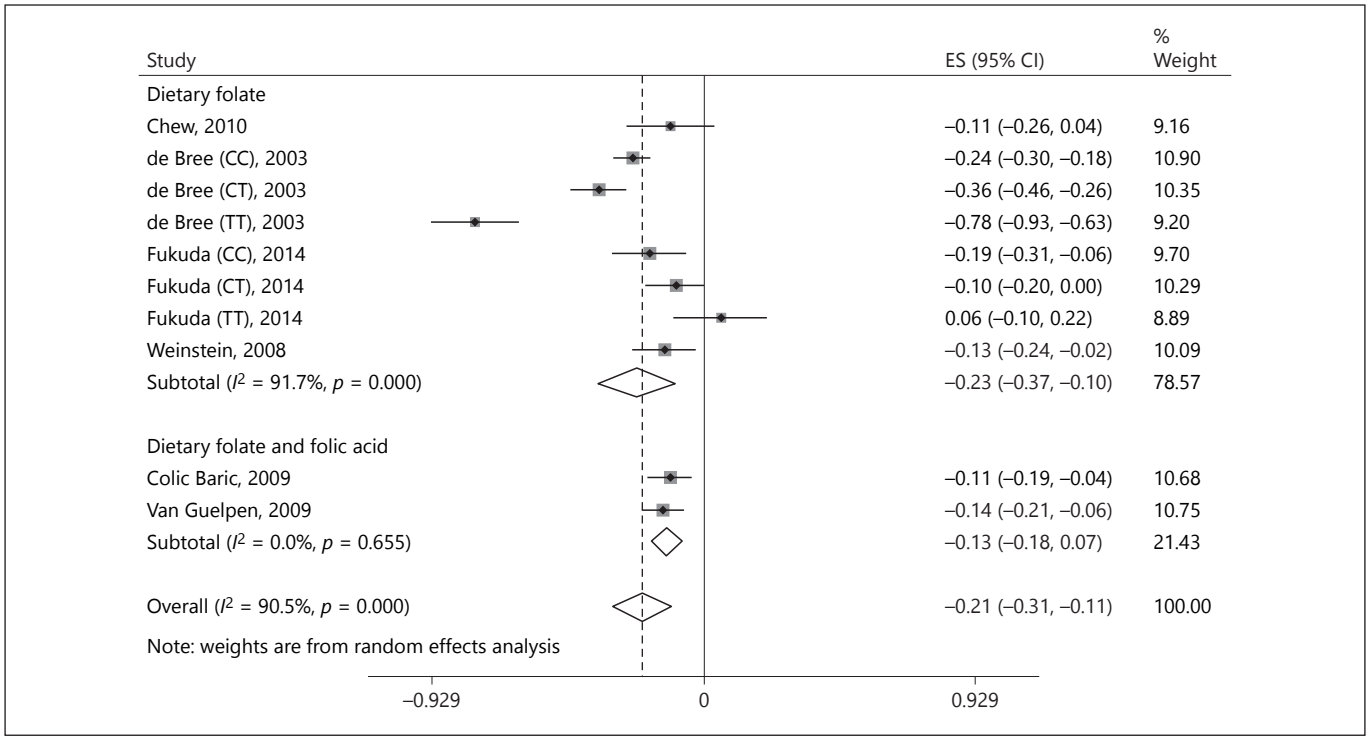

Fig. 4. Random effects meta-analysis of the association between dietary folate and plasma homocysteine folate in adults and elderly, stratified for the matrix of provided folate. The regression coefficients (β) represent the linear association between $\log_e$ transformed folate intake and $\log_e$ transformed plasma homocysteine folate status (lines represent the confidence intervals of each study). "Dietary folate" shows natural food folate expressed in μg/day in all studies; "Dietary folate including folic acid" shows natural food folate plus folic acid from supplements and fortified foods expressed in μg/day in one study [25], whereas in another [14] it was expressed in DFE (Table 3).

The mean age of the study participants (continuous variable) was a statistically significant determinant of the overall association (p = 0.004).

We did not perform stratified meta-analyses or meta-regression for the dietary intake method and total homocysteine because of the small number of studies in one of the strata. Also, stratified meta-analyses or meta-regression for the analytical method was not possible, as all studies used non-microbiological assay to determine plasma homocysteine levels.

The association between total folate intake (folic acid from supplements and/or fortified foods plus dietary folate) and its biomarkers in plasma/serum, RBCs, and plasma homocysteine levels in adults and elderly, stratified by folate matrix for plasma/serum, RBCs, and plasma homocysteine, and by dietary intake method and analytical method used to measure folate status biomarker for plasma/serum and RBCs is summarized in Table 4.

Discussion

This systematic review gives a summary of available observational data on the association between folate intake and its biomarkers for adults and elderly. It uses best quality evidence from a large number of subjects with a broad range of folate intakes as from natural settings, and focuses on EURRECA proposed best practice biomarkers of folate status [5]. The main association found by pooling data from observational studies showed that total folate intake (i.e., folic acid plus dietary folate) in the range of 152–662 μg/day is significantly associated with folate status biomarkers: serum/plasma folate, RBC folate, and total plasma homocysteine concentrations in adults and elderly. For every doubling in folate intake, the changes in serum/plasma folate, RBC folate, and plasma homocysteine were +22, +21, and –16% respectively. This information, along with estimates from RCTs [3] can be used to decide what dose of folate or folic acid to recommend for optimal intake among adults and elderly.

Table 4. Associations between total folate supply (i.e., natural food folate plus folic acid from supplements and/or fortified foods) and folate in plasma/serum and in red blood cells, as well as on plasma homocysteine levels in adults and elderly, stratified by folate matrix, dietary intake method, and analytical method used to measure folate status

Stratum for analysis	Folate in serum/plasma			Folate in red blood cells			Plasma homocysteine		
	number of estimates (*n* participants)	regression coefficient[1] (95% CI)	heterogeneity I^2 (%)	number of estimates (*n* participants)	regression coefficient[1] (95% CI)	heterogeneity I^2 (%)	number of estimates (*n* participants)	regression coefficient[1] (95% CI)	heterogeneity I^2 (%)
All studies	26 (8,344)	0.29 (0.21–0.37) $p < 0.0001$	92	13 (3,997)	0.28 (0.21–0.36) $p < 0.0001$	88	10 (3,375)	–0.21 (–0.31 to –0.11) $p < 0.0001$	91
Folate matrix									
Diet (folate μg/day)	16 (6,139)	0.33 (0.22–0.44) $p < 0.0001$	92	6 (1,131)	0.31 (0.17 p 0.45) $p < 0.0001$	76	8 (2,983)	–0.23 (–0.37 to –0.10) $p < 0.0001$	92
Diet and supplements (folate μg/day)	10 (2,205)	0.22 (0.12–0.33) $p < 0.0001$	87	7 (2,866)	0.27 (0.17–0.36) $p < 0.0001$	92	2 (392)	–0.13 (–0.18 to –0.07) $p < 0.0001$	0
Dietary intake method									
Food Frequency Questionnaire	17 (7,391)	0.33 (0.22–0.43) $p < 0.0001$	94	4 (885)	0.16 (0.12–0.19) $p = 0.003$	0			
24-h recall and food record	9 (953)	0.18 (0.10–0.25) $p = 0.002$	39	9 (3,112)	0.33 (0.25–0.41) $p < 0.0001$	82			
Analytical method									
Microbiological assay	9 (4,792)	0.51 (0.37–0.64) $p < 0.0001$	87	3 (1,299)	0.30 (0.11–0.49) $p < 0.0001$	92			
Non-microbiological assay	17 (3,552)	0.18 (0.14–0.22) $p < 0.0001$	49	10 (2,698)	0.27 (0.19–0.35) $p < 0.0001$	82			

[1] Regression coefficients for the linear association between $\log_e$ transformed folate intake and $\log_e$ transformed folate status biomarkers.

For the interpretation of the results, it should be acknowledged that the studies included in this meta-analysis differed in terms of study populations and methods. For serum/plasma folate and RBC folate, the mean age was not a clear predictor of the association. Although the folate food matrix (for all 3 biomarkers), dietary assessment method (for RBC folate), and analytical method used to measure the folate status (for serum/plasma folate and RBC folate) affected the dose-response relationship, this explained only part of the large heterogeneity (I^2 ranged from 88 to 92%) between the studies. Regarding the food matrix, we observed a stronger association for the estimate based on studies that included diet alone in comparison to those in which folate supply was from diet and supplements. This finding is in line with results from a similar systematic review of RCTs among women in childbearing age and in pregnancy and lactation [29]. Because synthetic folate is 1.7 times more bioavailable than naturally occurring folate [14, 37], total dietary folate should be expressed as DFE to account for this difference in bioavailability. However, for studies that assessed folate intake from the diet including the use of supplements, this conversion into DFE was done in only 5 out of 12 studies [14, 16, 22, 23, 32]. Also, some studies used 100–200 μg/day of folic acid as a conservative estimate of intake from supplements based on supplements available on the market, and added that amount without conversion to DFE to total dietary folate [18, 19, 25]. Finally, the proportion of regular and occasional supplement users varied between 4.8 and 26%, with average supplement dose up to 200 μg/day [11, 12, 17, 18, 22, 25, 27, 29]. This fact along with the lack of standardization in reporting total folate intake as DFE will have affected the estimates and contributed to heterogeneity of stratum-specific estimates. In addition, the stronger folate intake-status association resulting from natural folate in comparison to that from natural folate and folic acid could be linked to the property of naturally occurring folate: reportedly, it is as effective as or even more effective than folic acid in preserving folate status [35].

Studies included in these meta-analyses used a variety of analytical methods for measurement of folate status markers: 7 for serum/plasma, 5 for RBC folate, and 5 for plasma homocysteine. Because there were a small number of studies that applied the same analytical method per stratum for each biomarker, we were able to perform stratified meta-analyses or meta-regression only for the broad categories of microbiological assay and non-microbiological assay. This approach was already followed in a meta-analysis of folic acid intake-status relationship using the data from RCT [3]. We found statistically sig-

nificant higher estimates for folate intake-status serum/plasma relationship when microbiological assay was used rather than non-microbiological assay, whereas the estimates for RBC folate did not statistically differ between the 2. Our finding that the type of analytical method had a considerable influence on the response, confirms the expert opinion that comparison of studies that used different assays requires caution, as different analytical methods are known to cause up to 30% difference in folate status results [38]. Indeed, future research should benefit from methodologically comparable data on folate intake and status.

We postulated that factors that explain heterogeneity in plasma/serum and RBC folate could be similar. Since plasma/serum and RBC folate yielded similar overall estimates, we combined the 22 estimates on serum/plasma folate and the 13 estimates on RBC folate in a meta-regression model, with the biomarker medium (serum/plasma or RBC folate) as an additional covariable. (Given that results from the same study are not statistically independent, we used studies [13, 14, 16, 23] to get the estimates for red blood folate only.) The biomarker medium in fact provided no statistically significant explanation ($p = 0.82$). Our interpretation of this observation is that similar factors cause heterogeneity for both biomarkers.

Studies that were included in this review applied diverse dietary assessment methods too. It is another aspect that should be taken into consideration when examining the heterogeneity between studies, as it is known that dietary intake measurement errors differ considerably by dietary assessment method [39]. For example, in general, FFQs are designed to rank individuals rather than to assess their absolute intake levels. Also, a small number of replicates of 24HDR with no adjustment for intra-individual variability can give different estimates in comparison to multiple replicates. The FFQ was the most commonly used dietary instrument in this review; it was used to reflect folate intake for up to 3 months or 1 year. Stratified meta-analysis for the dietary intake method in serum/plasma and in RBC folate gave different estimates between the FFQ subgroup and 24HDR or FR subgroup: the association in FFQ subgroup was statistically and significantly weaker for RBC folate and statistically and not significantly stronger in plasma/serum folate when compared to 24HDR or FR subgroups per each biomarker. Observed differences in estimates indicate varying levels of bias associated with each measurement tool, for example, underreporting for 24 h recall, over- or underreporting of important folate-containing foods by the FFQ, inadequate folate values in food tables, different bioavailability of folate from different food products, the seasonality of data collection, or perhaps a combination of these conditions [40, 41]. For example, the nutrient intakes as from 24HDR is adjusted to reflect long-term exposure and, therefore, they may be more comparable to status assessed by RBC folate than by serum folate [42] as it implies from the results in this study. We hypothesized that, to some extent, the reason for the high heterogeneity in the meta-analysis of serum/plasma folate for the studies with FFQ might be genetic variation in the 677C→T mutation in the gene, which is known to significantly influence concentrations of folate status biomarkers at folate intake levels <250 μg/day [15]. Because 2 studies provided the information on this gene mutation, we performed sensitivity analyses by removing the estimates with TT genotypes [15, 17]; however the heterogeneity remained the same ($I^2 = 94\%$). We also acknowledge that the estimation of folate intake requires reliable data on food composition. An important factor that hampers comparability of the data (between studies and across countries) and can be a source of errors is the lack of uniformity in food composition databases. This issue has been addressed in many past and ongoing projects worldwide. For instance, an important conclusion derived from the EFCOSUM project was that it is not possible at the present time to make existing food consumption data comparable at the nutrient level [45]. Therefore, for the purpose of this study, a problem of application of different food composition databases could not have been solved. Overall, when the pooled estimates from stratified subgroup analysis are analyzed in detail, folate matrix is the influencing factor on the overall beta for each folate-specific biomarker, in addition to the type of analytical method for serum/plasma folate (we were unable to do stratified analyses for dietary intake assessment and the type of analytical method at homocysteine because of small study numbers in each subgroup).

The relationship between folate intake and status in adults and the elderly has also been assessed in a meta-analysis of RCTs [3]. The association in RCTs was stronger than that in the observational studies in this paper, that is, for every doubling in folic acid intake, the increase in serum/plasma folate and RBC folate was 63 and 31% respectively. Another publication by Berti et al. [35] based on RCTs in women of reproductive age showed relationships for folate in RBCs and for total plasma homocysteine similar to those reported in the present study: 0.33 (95% CI 0.23–0.44) and –0.12 (95% CI –0.15 to –0.08), respectively, whereas the association for serum/plasma folate was stronger – 0.65 (95% CI 0.39–0.93) – than that shown by our data. One potential ex-

planation for larger β in the RCTs could be higher bioavailabity of folic acid (85% or greater), which appears unaltered in the circulation and gives rise to serum/plasma folate in comparison to natural folates (25–50%) [43]. On the other hand, our estimates for serum/plasma as from subgroup analyses from microbiological assay are similar to those from RCTs. Assuming that microbiological assay is considered the gold standard for total folate measurement in serum [3], it may be that the overall pooled estimate for serum/plasma as from this paper is underestimated.

The observed mean folate intake in this review (266 μg/day) is in line with intakes indicated by individual observational studies that assessed folate intake in adults and elderly in Europe: the mean folate intake for adults and elderly in the European Nutrition and Health Report (25 countries) ranged from 130 to 370 μg/day in women and from 150 to 440 μg/day in men [44], whereas the results from the EPIC cohort (10 countries) reported 200–300 μg/day in women and 250–350 μg/day in men [45]. The levels of serum/plasma folate reported in a recent publication on micronutrient intake and status is Europe were 14–23 nmol/L [46], and in the publication by Duffy et al. [3], the levels were in the range 6–24 nmol/L and this is in line with the range observed in this review (5–31 nmol/L).

Thus, mean levels of intake and biomarkers are comparable to other studies, whereas their associations are heterogeneous. Although this heterogeneity might partially derive from methodological factors (standardization to DFE, dietary assessment method, analytical method used to determine level of status biomarkers), the overall estimates are consistent with those of other similar dose-response meta-analyses of trials (using synthetic folate) and observational studies among pregnant women. Possibly, to some extent, part of the between-study heterogeneity might reflect true differences between populations that average out in the meta-analysis.

Conclusion

This study shows that the quantified statistically significant relationships between folate intake and folate status biomarkers are similarly positive for serum/plasma and RBC folate and inverse for homocysteine. Because the ranges of dietary folate intake and serum/plasma folate shown in this review are similar to those reported in other studies conducted involving adults and elderly, the estimates shown by this review reflect folate intake and serum/plasma folate relationship in these population groups. The relative heterogeneity that we found between the studies indicates that for getting more reliable estimates, future research should take into consideration: (i) known differences in bioavailability between the natural forms and folic acid from supplements and fortified foods and (ii) variability that exists among different folate assay methodologies and across different laboratories [3]. Nevertheless, this study uses best quality available data and can be used to study the relationships between folate intake, status and health-related outcomes more in depth. Furthermore, reported estimates as from this paper together with those from RCTs can serve as a basis for further modeling toward determining dietary reference values for folate, such as the Average Nutrient Requirement (ANR), which vary greatly across countries (200–320 μg/day) [47–49]. The principle of that methodology has recently been developed within the EURRECA NoE and is readily available [6].

Disclosure Statement

The authors have no relevant conflicts of interests to declare.

Acknowledgments

Funding and Sponsorship: This study has been carried out within the EURRECA Network of Excellence (http://www.eurreca.org), which is financially supported by the Commission of the European Communities, specific Research, Technology and Development (RTD) Programme Quality of Life and Management of Living Resources, within the Sixth Framework Programme, project No. FP6 036196-2. This report does not necessarily reflect the Commission's views or its future policy in this area. This work was in part funded by the Ministry of Education and Science of Republic Serbia, project III 41030.

Author Contributions

R.N. conceptualized the paper, contributed to the literature searches and data extraction, performed the statistical analyses, and drafted the manuscript. P.V.V., D.R.-M., M.N. and A.G. supervised the work and assisted with analysis and editing of the manuscript. O.W.S., H.M.N., M.D., L.H., C.D. and J.M.S.R. contributed to the literature searches and data extraction. M.G., M.G., L..C.P.G.M.G., and P.V.V. were involved in development of the study hypothesis, editing the working versions of the manuscript and provided advice regarding interpretation of the results. All authors participated in the writing or editing of the draft versions of the article and have read and approved of the final version of the submitted manuscript.

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