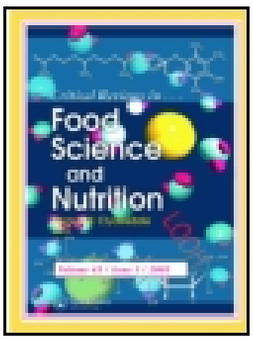

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Intake of vitamin B6, folate, and vitamin B12 and risk of coronary heart disease: a systematic review and dose-response meta-analysis of prospective cohort studies

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REVIEW

Intake of vitamin B6, folate, and vitamin B12 and risk of coronary heart disease: a systematic review and dose-response meta-analysis of prospective cohort studies

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ABSTRACT

The objective of this study was to quantify the association of B-vitamins intake with the future risk of coronary heart disease (CHD). A systematic search was performed with the use of PubMed and Scopus from inception to April 30, 2018. Prospective cohort studies evaluating the association of intake of folate, vitamin B6, and vitamin B12 with risk of CHD in the general population were included. A random-effects meta-analysis was performed. Eleven prospective cohort studies (total $n = 369{,}746$) with 5133 cases of CHD were included in the analyses. The relative risks were: 0.79 (95%CI: 0.69, 0.89; $I^2 = 67\%$) for a 250 μg/d increment in folate intake; 0.87 (95%CI: 0.78, 0.96; $I^2 = 80\%$) for a 0.5 mg/d increment in vitamin B6 intake; and 0.97 (95%CI: 0.80, 1.14: $I^2 = 67\%$) for a 3 μg/d increment in vitamin B12 intake. The results did not change materially when the analyses were restricted only to dietary vitamins intake. A nonlinear dose-response meta-analysis demonstrated a linear inverse association between folate and vitamin B6 intake and risk of CHD. In conclusion, higher intake of folate and vitamin B6 is associated with a lower risk of CHD in the general population.

KEYWORDS

Coronary heart disease; folic acid; meta-analysis; vitamin B6

Introduction

Cardiovascular diseases (CVD) are still the leading causes of death worldwide, in a way that about 32% of deaths were attributable to CVD in 2013 (GBD 2015). Of those, about 40% were attributable to coronary heart disease (CHD) (Roth et al. 2015). Homocysteine, a sulfur-containing amino acid, mainly through increasing blood pressure and inducing thrombosis and atherogenesis (Lentz 2005), is associated with a higher risk of developing CVD (HSC 2002), and its elevated plasma concentration is considered a risk factor for CVD.

B-vitamins including vitamin B6, folate, and vitamin B12 have important functions in one-carbon units metabolic pathways and thereby are associated with homocysteine metabolism (Ntaios et al. 2009; Scott and Weir 1998). Homocysteine through vitamin B12- and folate-dependent pathways can be remethylated to methionine, or via vitamin B6-dependent pathways can be converted to cysteine (Ntaios et al. 2009). Several interventional studies have presented convincing evidence that supplementation with B-vitamins (Huang et al. 2012; Thambyrajah et al. 2000; van Oort et al. 2003), or supplementation with foods rich in, or fortified with B-vitamins (Broekmans et al. 2000; Brouwer et al. 1999; Chait et al. 1999; Jang et al. 2001) can reduce the circulating levels of homocysteine. Despite this property, interventional studies have failed to show that supplementation with B-vitamins can decrease the risk of CHD (Bazzano et al. 2006; Bonaa et al. 2006; Clarke et al. 2010; Ebbing et al. 2008; Li et al. 2016; Lonn et al. 2001; Toole et al. 2004). High-dose supplementations, short term follow-up durations, and prior history of CVD or other chronic diseases are some of the proposed explanations about the null findings in interventional studies.

However, evidence from observational studies in this regard is scarce. To our knowledge, only one meta-analysis of seven prospective cohort studies showed that an increase of 200 μg/d in folate intake was associated with a 12% lower risk of CHD (Wang et al. 2012). But, the shape of the dose-response relation was not determined. In addition, there is no summarized evidence regarding other vitamins including vitamin B6 and vitamin B12. It is estimated that about 20% of all cases of CHD in the US are attributable to poor diet quality including low intake of fruit, vegetables, dairy products, and whole grains (Mozaffarian et al. 2015). In addition, it has been proposed that CHD may be a deficiency disease (Klevay 2004). Therefore, investigating the possible association of usual dietary intake of B-vitamins with risk of CHD in the general population may provide additional evidence regarding the possible role of dietary factors in the etiology

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and pathophysiology of CHD. Thus, the objective of this study was to quantify the association of dietary intake of folate, Vitamin B6, and vitamin B12 with risk of CHD in the general population, with the use of prospective observational studies.

Methods

This systematic review has been reported according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) checklist (Stroup et al. 2000).

Search strategy

A systematic literature search was performed with the use of PubMed and Scopus, from their inception to April 30, 2018. The following combination of key words was used to identify studies from electronic databases: ["folate" OR "folic acid" OR "B6" OR "pyridoxine" OR "B12" OR "cobalamin" OR "vitamin" OR "B-vitamins"] AND ["mortality" OR "death" OR "cardiovascular" OR "CVD" OR "coronary heart disease" OR "CHD" OR "coronary artery disease" OR "CAD" OR "stroke" OR "myocardial infarction" OR "MI" OR "coronary event"] AND ["prospective" OR "prospectively" OR "retrospective" OR "retrospectively" OR "Cohort" OR "Cohorts" OR "Longitudinal" OR "observational" OR "Observation" OR "Follow-up" OR "Nested" OR "case-control" OR "case-cohort" OR "case-referent" OR "relative risk" OR "RR" OR "odds ratio" OR "hazard ratio" OR "HR" OR "risk"]. The reference lists of retrieved articles and relevant reviews also were manually searched. The search was restricted to articles published in English.

Eligibility and study selection

Two authors (AJ, MSZ) reviewed the titles and abstracts of all studies identified, and selected prospective observational studies which (1) were conducted among adults aged 18 years or older; (2) reported intake of folate, vitamin B6, or vitamin B12 as exposure and in at least two categories; (3) reported the outcome of interest as total CHD incidence or mortality including myocardial infarction, CHD, and other coronary events; and (4) reported risk estimates (relative risk (RR) or hazard ratio or odds ratio) and their corresponding 95% confidence interval of CHD for each category of B-vitamins intake. Studies that reported exposures in at least three quantitative categories, and reported the numbers of cases and participants/person-years or non-cases in each category of dietary B-vitamins were considered eligible for inclusion in dose-response analyses. If more than one publication of a given study exists, only the publication with higher number of participants was included.

Data extraction and quality assessment

Two independent investigators (AJ, MSZ) extracted the following information from eligible studies: first author's name, publication year, study name, country, age range and/or mean age (year), number of participants/cases, dietary assessment method, exposure levels, effect size, and covariates adjusted in the multivariate analysis. The most comprehensive covariate adjustments model was selected from each study. We used the Newcastle-Ottawa Scale to assess the quality of primary studies (Stang 2010). Furthermore, we applied the NutriGrade scoring system (a maximum of 10 points) for judgment about the quality of meta-evidence (Schwingshackl et al. 2016).

Statistical analysis

The relative risk (RR) and 95%CI was considered as the effect size of all studies. The reported hazard ratios were considered as equal as RR. For studies that reported effect estimates as odds ratio, we converted effect estimates to risk ratio (Zhang and Yu 1998). For the highest versus lowest category meta-analysis, the reported risk estimates for the highest compared with the lowest categories of B-vitamins intake were combined using the DerSimonian and Laird random-effects model (DerSimonian and Laird 1986). If a given study reported the results for men and women or other subgroups separately, we combined the effect estimates using a fixed-effects model and used the combined effect size for meta-analysis. To test the potential influence of each study on pooled effect size, sensitivity analyses were performed with the sequential exclusion of each study at a time. Subgroup analyses were performed by some of the study and participant's characteristics. Between-studies heterogeneity was explored using Cochrane's Q test of heterogeneity and I^2 statistic ($P < 0.05$) (Higgins et al. 2003). The potential publication bias was assessed using funnel plots asymmetry and tested by Egger's asymmetry test (Egger et al. 1997) and Begg's test ($P < 0.10$) (Begg and Mazumdar 1994).

We measured the linear dose-response relation using a generalized least squares trend estimation, according to the methods developed by Greenland and Longnecker (Berlin, Longnecker, and Greenland 1993; Orsini, Bellocco, and Greenland 2006). This method needs distribution of cases and participants/person-years or non-cases and adjusted RR and its 95%CI across categories of B-vitamins intake. Study-specific results were combined using a random-effects model. The RR and its 95%CI were calculated for a 250 µg/d increment in folate intake, a 0.5 mg/d increment in vitamin B6 intake, and for a 3 µg/d increment in vitamin B12 intake. If the numbers of participants/cases or person-years have not been reported in the primary studies, we estimated them by dividing the total number of participants/cases or person-years by the number of categories, if the exposure was defined as quantiles. A potential non-linear association was examined by modeling dietary B-vitamins intake levels using restricted cubic splines with three knots at fixed percentiles (10, 50, and 90%) of the distribution (Orsini et al. 2012). A P-value for non-linearity of the meta-analysis was calculated by testing the null hypothesis that the coefficient of the second spline was equal to zero. All analyses were conducted

with Stata software, version 13 (Stata Corp, College Station, Texas, USA). A *P*-value <0.05 was considered statistically significant.

Results

The initial systematic literature search identified 5108 publications, plus two publications through hand searching. Of these, 393 publications were duplicates and another 4639 publications were eliminated on the basis of screening of the title and abstract. Finally, 78 full text articles were assessed for inclusion in the present meta-analysis; on these, another 68 publications were excluded which respective reasons for study exclusion are detailed in Fig. 1. Finally, 10 publications were considered eligible for inclusion in the present meta-analysis (Cui et al. 2010; Dalmeijer et al. 2008; Drogan et al., 2006; Ishihara et al. 2008; Luu et al. 2011; Marniemi et al. 2005; Rimm et al. 1998; Van Guelpen et al. 2009; Voutilainen et al. 2001; Zhao et al. 2018). One publication reported the results of the two separate large-scale prospective cohort studies (Zhao et al. 2018), and was regarded as two separate studies. Thus, 11 studies with a total of 369,746 participants and 5133 cases of CHD were included in the final analyses. Two studies were from the US (Luu et al. 2011; Rimm et al. 1998), five from Europe (Dalmeijer et al. 2008; Drogan et al. 2006; Marniemi et al. 2005; Van Guelpen et al. 2009; Voutilainen et al. 2001), and four studies (three publications) were from Asia (Cui et al. 2010; Ishihara et al. 2008; Zhao et al. 2018). Three studies included only women (Dalmeijer et al. 2008; Rimm et al. 1998; Zhao et al. 2018), two studies included only men (Voutilainen et al. 2001; Zhao et al. 2018), and the remainders included both sexes. One study had a nested case-referent design (Van Guelpen et al. 2009), and the remainders were prospective cohort studies. Follow-up durations ranged between 4.5 and 16.5 years. One study used a 4-day food record (Voutilainen et al. 2001), one study performed dietary history interview (Marniemi et al. 2005), and other studies used a food frequency questionnaire to assess dietary intake. Of the 11 studies included in the present meta-analysis, most studies controlled for body mass index (BMI) ($n = 10$), smoking ($n = 10$), and history of diabetes ($n = 9$); some of the studies controlled for alcohol intake ($n = 7$), energy intake ($n = 7$), physical activity ($n = 6$), and intake of

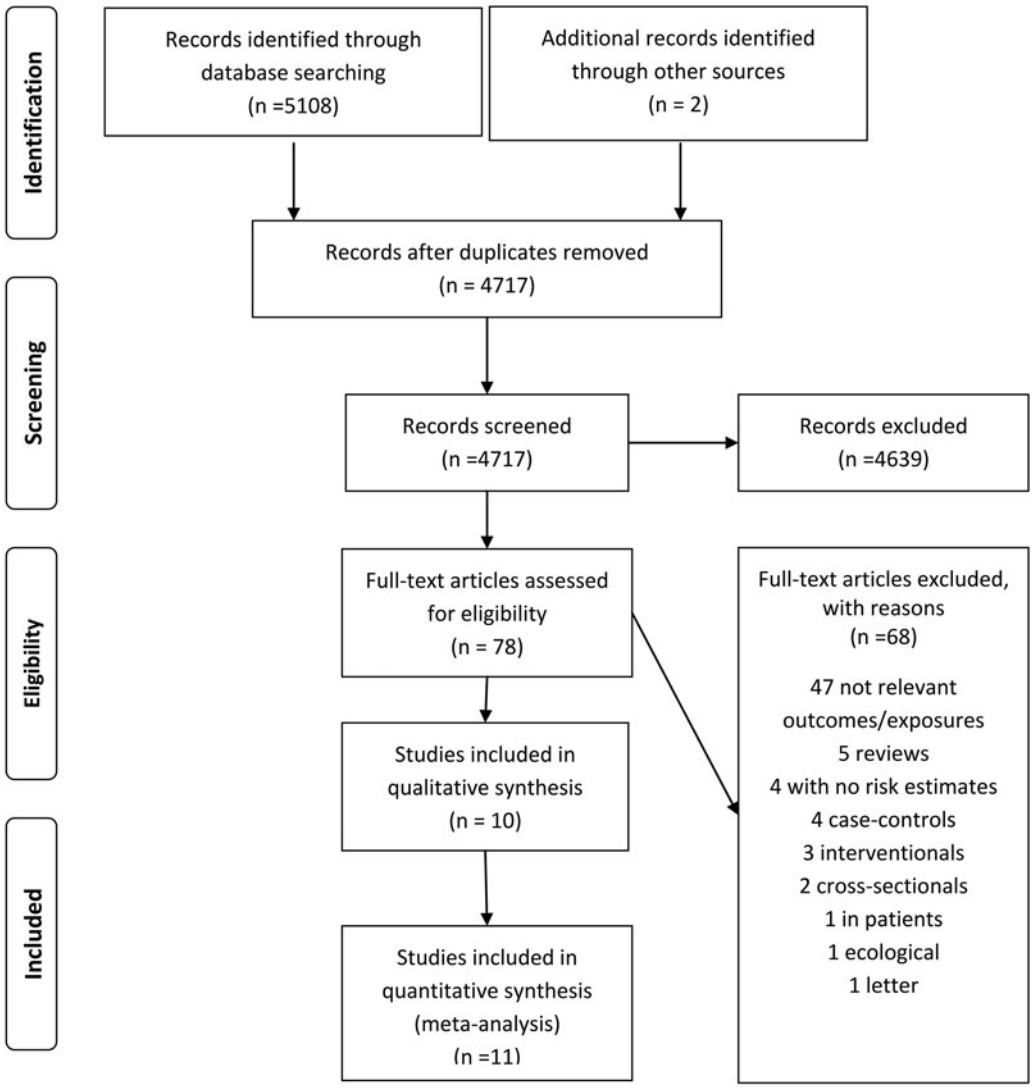

Figure 1. Literature search and study selection process for inclusion in meta-analysis of B-vitamins and risk of coronary heart disease.

saturated fatty acids ($n = 6$); and a few studies controlled for intake of polyunsaturated fatty acids ($n = 5$). The general characteristics of the studies are presented in Table 1, and the number of participants/cases and reported risk estimates of CHD across categories of B-vitamins intake in each study are provided in Supplementary Table 1.

Folate intake and risk of CHD

Nine prospective cohort studies (total $n = 235{,}284$) with 4281 cases were included in the analysis of folate intake and risk of CHD (Cui et al. 2010; Dalmeijer et al. 2008; Drogan et al. 2006; Ishihara et al. 2008; Luu et al. 2011; Marniemi et al. 2005; Rimm et al. 1998; Van Guelpen et al. 2009; Voutilainen et al. 2001). The relative risk of CHD for the highest compared with the lowest category of folate intake was 0.69 (95%CI: 0.57, 0.81), with moderate evidence of heterogeneity, $I^2 = 54\%$, 95%CI: 0%, 79%; $P_{\text{heterogeneity}} = 0.03$ (Fig. 2a). The relative risk was 0.68 (95%CI: 0.53, 0.84; $I^2 = 63\%$, $n = 7$ studies) when only dietary intake was taken into account, and was 0.73 (95%CI: 0.59, 0.87; $I^2 = 0\%$, $n = 3$ studies) when intakes from both food and supplements were taken into consideration.

The association did not change materially with the sequential exclusion of each study at a time (RR ranged between 0.67 and 0.73). In the sensitivity analysis, the Kuopio Ischemic Heart Disease Risk Factor Study (Voutilainen et al. 2001), the study that considered acute coronary events as the outcome of interest, explained much of the observed heterogeneity in the data (RR: 0.73, 95%CI: 0.65, 0.81; $I^2 = 0.4\%$). Only one study did not exclude participants with a history of CVD (Marniemi et al., 2005), and a sensitivity analysis with the exclusion of this study demonstrated similar result with the main analysis (RR: 0.67, 95%CI: 0.56, 0.78; $I^2 = 50\%$).

In the subgroup analyses, an inverse association remained significant even after adjustment for main confounders including BMI, smoking, physical activity, alcohol consumption, history of diabetes, and intakes of energy, saturated, and unsaturated fats (Table 2). The subgroup analyses

Table 1. General characteristics of the studies included in meta-analysis of B-vitamins and risk of coronary heart disease.

Author name, publication year	Study name, country	Age (range, mean; years)	Follow-up duration (years)	Participants/ cases	Gender	Exposure (s)	Dietary assessment	Outcome	Quality score (max. 9 points)
Cui, 2010	Japan Collaborative Cohort Study, Japan	40–79 (56)	14	58,730/424	W/M	B6, folate, B12	FFQ	CHD	9
Dalmeijer, 2008	PROSPECT–EPIC cohort, Netherland	49–70 (57)	8	16,165/493	W	Folate	FFQ	CHD	9
Drogan, 2006	European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study, Germany	35–64 (50)	4.6	22,245/129	W/M	Folate	FFQ	MI	7
Van Guelpen, 2009	Northern Sweden Health and Disease Study, Sweden	25–74	13	1004/247	W/M	B6, folate, B12	FFQ	MI	8
Ishihara, 2008	Japan Public Health Center-Based Prospective Study Cohort I, Japan	40–59 (49)	11.5	40,803/251	W/M	B6, folate, B12	FFQ	CHD	8
Luu, 2011	Atherosclerosis Risk in Communities Study, US	45–64 (54)	16.5	13,520/1469	W/M	Folate	FFQ	CHD	8
Marniemi, 2005	Turku health survey, Finland	65–99 (79)	10	755/130	W/M	B6, folate, B12	Dietary history interview	MI	6
Rimm, 1998	Nurses' Health study, US	30–55 (46)	14	80,082/939	W	B6, folate	FFQ	CHD	8
Voutilainen, 2001	Kuopio Ischemic Heart Disease Risk Factor Study, Finland	42–60	10	1980/199	M	B6, folate, B12	4-day food record	ACE	8
Zhao, 2018	Shanghai Men's Health Study, China	40–74 (55)	10.3	59,746/422	M	B6	FFQ	CHD	8
Zhao, 2018	Shanghai Women's Health Study, China	40–70 (53)	16.2	74,734/430	W	B6	FFQ	CHD	8

Abbreviations: ACE, acute coronary event; CHD, coronary heart disease; FFQ, food frequency questionnaire; M, men; MI, myocardial infarction; W, women.

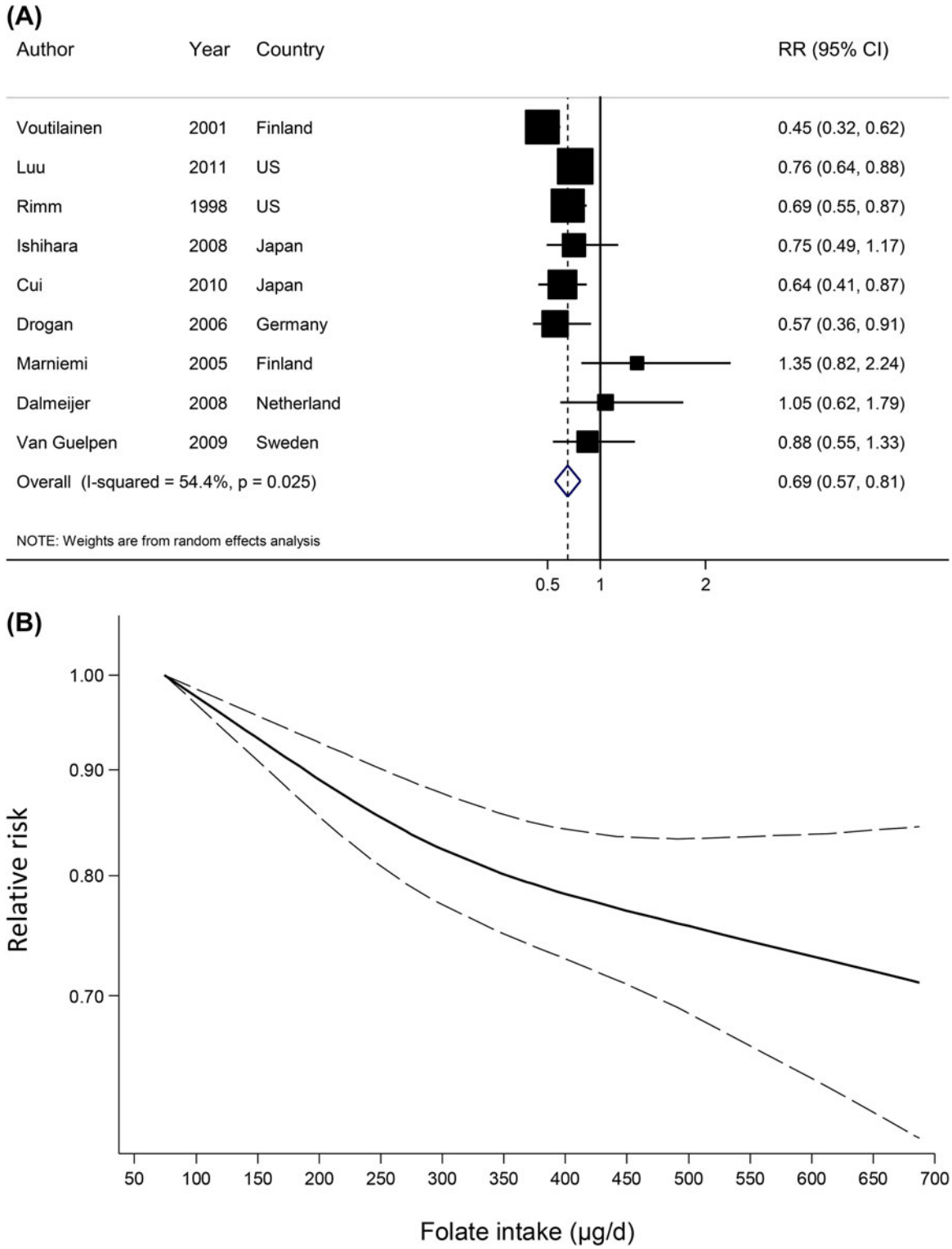

Figure 2. (a) Relative risk of coronary heart disease for the highest compared with the lowest category of folate intake. RR: relative risk. (b) Dose-response association between folate intake and risk of coronary heart disease.

suggested that geographical location, follow-up duration, number of participants, and adjustment for main confounders were potential sources of the observed heterogeneity in the data. There was no evidence of publication bias with Egger's test ($P = 0.72$) and Begg's test ($P = 0.25$) (Supplementary Figure 1).

Seven studies reported sufficient information for inclusion in the dose-response analysis (Cui et al. 2010; Dalmeijer et al. 2008; Ishihara et al. 2008; Luu et al. 2011; Rimm et al. 1998; Van Guelpen et al. 2009; Voutilainen et al. 2001). The linear trend estimation exhibited that an increase of 250 μg/d in folate intake was associated with a 21% lower risk of CHD (RR: 0.79, 95%CI: 0.69, 0.89; $I^2 = 67\%$) (Supplementary Figure 2). There was a linear inverse association between folate intake and risk of CHD (P for nonlinearity =0.07) (Fig. 2b).

Table 2. Subgroup analyses of folate intake and risk of coronary heart disease (high *vs.* low analysis).

		n	RR (95%CI)	I^2(%), $P_{heterogeneity}$ [1]	$P_{between}$ [2]
All studies		9	0.69 (0.57–0.81)	54%, 0.03	–
Folate source					
Food only		7	0.68 (0.53–0.84)	63%, 0.01	0.35
Food and supplements		3	0.73 (0.59–0.87)	0%, 0.62	
Sex					
Men		2	0.55 (0.30–0.81)	61%, 0.11	0.01
Women		3	0.69 (0.55–0.83)	1%, 0.36	
Both		5	0.76 (0.62–0.89)	17%, 0.30	
Geographical region					
US		2	0.73 (0.64–0.83)	0%, 0.49	0.10
Europe		5	0.74 (0.47–1.01)	67%, 0.02	
Asia		2	0.67 (0.48–0.87)	0%, 0.60	
Outcome					
CHD incidence		8	0.70 (0.56–0.84)	0.60%, 0.01	0.79
CHD death		1	0.64 (0.41–0.87)	–	
Follow-up duration					
≤10 years		3	0.91 (0.42–1.39)	63%, 0.07	0.58
>10 years		6	0.67 (0.54–0.79)	58%, 0.04	
Number of participants					
<20,000		5	0.77 (0.53–1.02)	76%, 0.002	0.92
>20,000		4	0.66 (0.55–0.78)	0%, 0.84	
Exclusion preexisting CVD	Yes	8	0.67 (0.56–0.78)	50%, 0.05	0.06
	No	1	1.35 (0.64–2.06)	–	
Adjustments					
Body mass index	Yes	8	0.67 (0.56–0.78)	50%, 0.05	0.06
	No	1	1.35 (0.64–2.06)	–	
Smoking	Yes	8	0.73 (0.65–0.81)	0.4%, 0.43	0.001
	No	1	0.45 (0.30–0.60)	–	
Alcohol consumption	Yes	5	0.68 (0.57–0.79)	0%, 0.65	0.84
	No	4	0.74 (0.48–1.00)	80%, 0.002	
Physical activity	Yes	5	0.73 (0.64–0.81)	0%, 0.57	0.03
	No	4	0.70 (0.43–0.97)	70%, 0.02	
History of diabetes	Yes	7	0.67 (0.53–0.81)	57%, 0.03	0.46
	No	2	0.93 (0.31–1.55)	68%, 0.08	
Energy intake	Yes	5	0.72 (0.63–0.82)	0%, 0.51	0.09
	No	4	0.71 (0.46–0.97)	73%, 0.01	
Intake of saturated fats	Yes	6	0.62 (0.49–0.74)	39%, 0.14	0.01
	No	3	0.84 (0.62–1.05)	29%, 0.24	
Intake of polyunsaturated fats	Yes	5	0.68 (0.57–0.79)	0%, 0.65	0.84
	No	4	0.74 (0.48–1.00)	80%, 0.002	

[1] *P*-heterogeneity within subgroups with the use of a random-effects model.
[2] *P*-heterogeneity between subgroups with the use of a fixed-effects model.
Abbreviations: CVD, cardiovascular disease; RR, relative risk.

Vitamin B6 intake and risk of CHD

Eight prospective studies (seven publications) comprising a total of 317,834 participants with 3042 cases were analyzes for the relation between vitamin B6 intake and risk of CHD (Cui et al. 2010; Ishihara et al. 2008; Marniemi et al. 2005; Rimm et al. 1998; Van Guelpen et al. 2009; Voutilainen et al. 2001; Zhao et al. 2018). Highest compared with the lowest category of vitamin B6 intake was associated with a 19% lower risk of CHD (RR: 0.81, 95%CI: 0.70, 0.93), with high heterogeneity, $I^2 = 70\%$, 95%CI: 37%, 84%; $P_{heterogeneity} = 0.001$ (Fig. 3a). The relative risk was 0.86 (95%CI: 0.74, 0.98; $I^2 = 66\%$, $n = 6$ studies) for dietary vitamin B6 intake, and was 0.68 (95%CI: 0.55, 0.85; $I^2 = 0\%$, $n = 3$ studies) for vitamin B6 from food and supplements.

In the sensitivity analysis, a significant inverse association persisted with the exclusion of each study in turn (RR ranged between 0.76 and 0.85). None of the excluded studies explained the large degree of the heterogeneity in the data. In the subgroup analyses, a significant inverse association persisted even after adjustment for BMI, smoking, physical activity, alcohol consumption, history of diabetes, and intakes of energy, saturated, and unsaturated fats (Supplementary Table 2). A strong inverse association was found among studies that excluded participants with preexisting CVD (RR: 0.70, 95%CI: 0.56, 0.83; I^2 =19%, $n = 6$ studies), but not among studies that included participants with CVD (RR: 0.93, 95%CI: 0.87, 1.00; $I^2 = 51\%$, $n = 2$ studies). The association was not significant among women, European studies, as well as among studies with shorter follow-up durations (≤10 years) (RR: 1.10, 95%CI: 0.36, 1.84; $n = 2$ studies) and lower number of participants (<20,000). Subgroup analyses suggested geographical location, and adjustments for fatty acids intakes as the potential sources of the heterogeneity. There was no evidence of publication bias with Egger's test ($P = 0.27$) and Begg's test ($P = 0.71$) (Supplementary Figure 3).

Six studies (five publications) were considered eligible for inclusion in the dose-response meta-analysis (Cui et al. 2010; Ishihara et al. 2008; Rimm et al. 1998; Van Guelpen et al. 2009; Zhao et al. 2018). A 0.50 mg/d increment in vitamin B6 intake was associated with a 13% lower risk (RR: 0.87, 95%CI: 0.78, 0.96; $I^2 = 80\%$) (Supplementary Figure 4). There was a linear inverse association between vitamin B6 intake and risk of CHD (P for nonlinearity =0.20) (Fig. 3b).

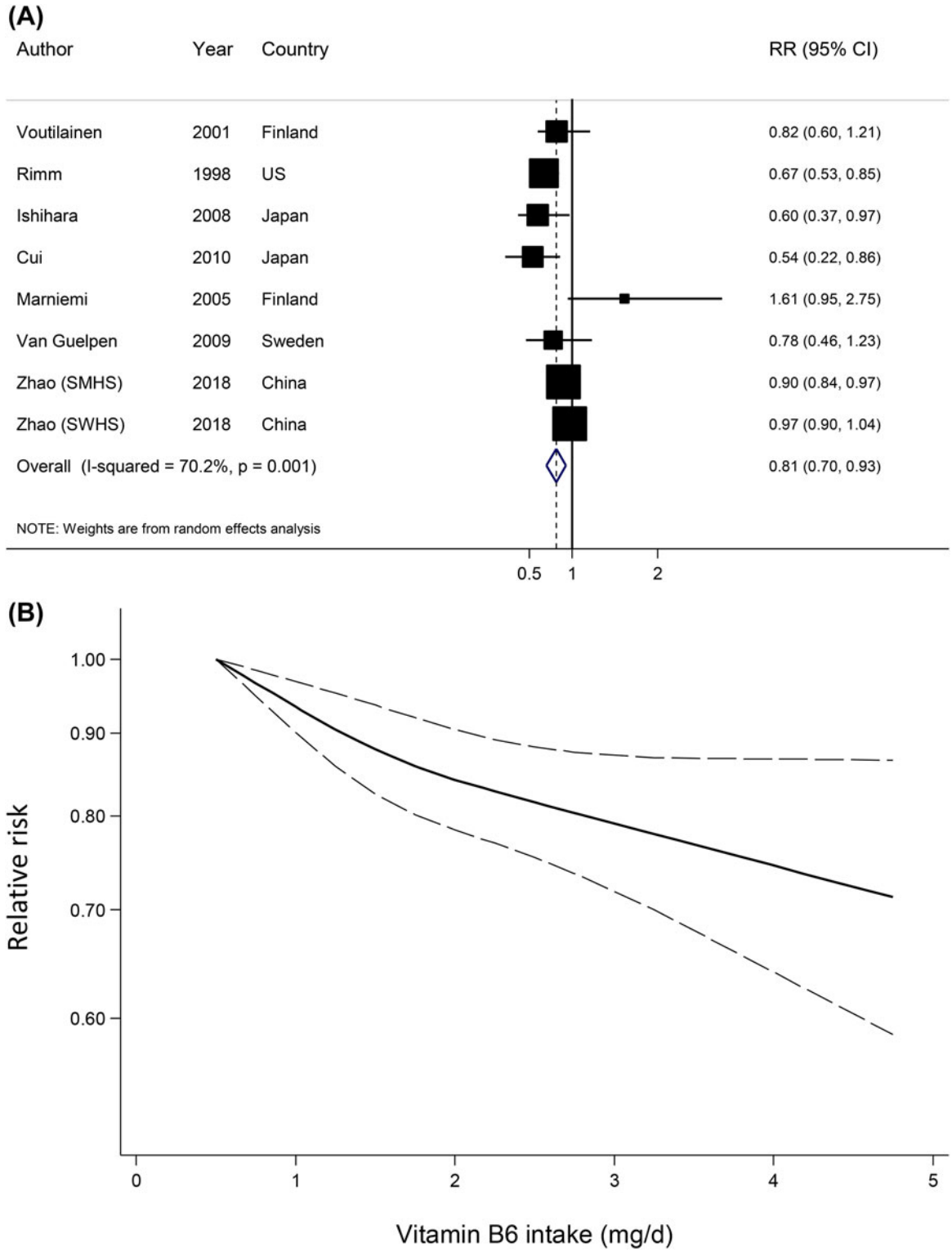

Figure 3. (a) Relative risk of coronary heart disease for the highest compared with the lowest category of vitamin B6 intake. RR: relative risk. SMHS: Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study. (b) Dose-response association between vitamin B6 intake and risk of coronary heart disease.

Vitamin B12 intake and risk of CHD

Five studies (total $n = 103{,}272$) with 1251 cases were identified on the association between vitamin B12 intake and risk of CHD (Cui et al. 2010; Ishihara et al. 2008; Marniemi et al. 2005; Van Guelpen et al. 2009; Voutilainen et al. 2001). All studies measured dietary vitamin B12 intake. Three studies reported a non-significant positive association, and two studies demonstrated an inverse association, which was statistically significant in one study. A non-significant inverse association was found for the highest compared with the lowest category of dietary vitamin B12 intake (RR: 0.97, 95%CI: 0.70, 1.25; $I^2 = 54\%$, 95%CI: 0%, 83%, $P_{\text{heterogeneity}} = 0.07$) (Fig. 4a), and for a 3 µg/d increment in dietary vitamin B12 (RR: 0.97, 95%CI: 0.80, 1.14; $I^2 = 67\%$, $P_{\text{heterogeneity}} = 0.05$; $n = 3$ studies) (Supplementary Figure 5). A nonlinear dose-

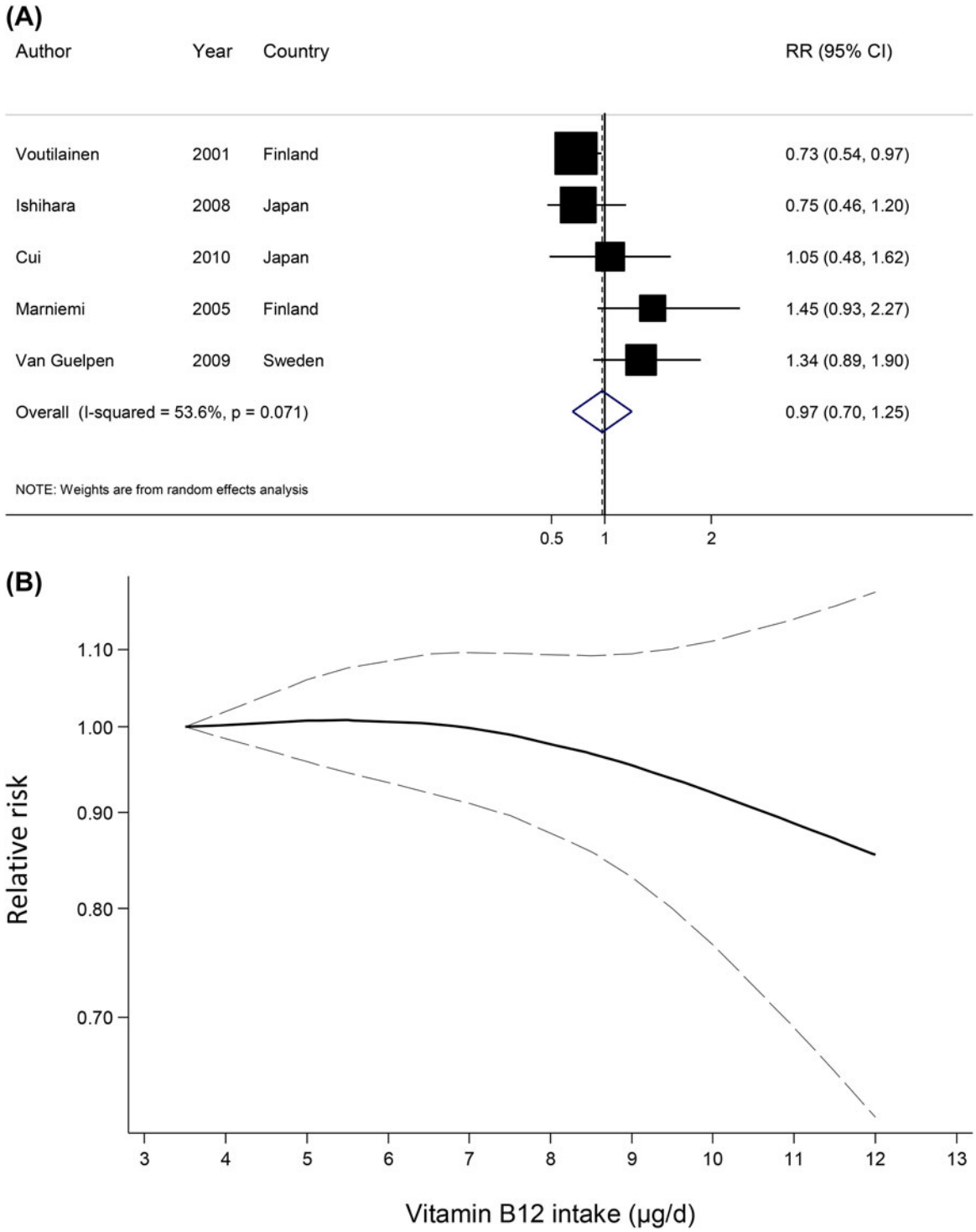

Figure 4. (a) Relative risk of coronary heart disease for the highest compared with the lowest category of vitamin B12 intake. RR: relative risk. (b). Dose-response association between vitamin B12 intake and risk of coronary heart disease.

response meta-analysis demonstrated that the risk of CHD did not change materially with increasing dietary vitamin B12 intake (P for nonlinearity =0.39) (Fig. 4b).

Quality of meta-evidence

All of the studies with the exception of one (Marniemi et al. 2005) were at high quality on the basis of the Newcastle-Ottawa Scale (≥7 scores). Also, the NutriGrade meta-evidence rating was "moderate" for folate (NutriGrade score: 7.9) and vitamin B6 (the NutriGrade score: 6.9), which suggests that there is moderate confidence in the effect estimate and further research may change the effect estimate. The NutriGrade meta-evidence rating was "low" for vitamin B12 (the NutriGrade score: 4.5)

Discussion

The present meta-analysis of prospective observational studies evaluated the association of B-vitamins intake with risk

of CHD, and exhibited that the risk of CHD decreased by 21% for each 250 µg/d increment in folate intake, and by 13% for each 0.5 mg/d increment in vitamin B6 intake. The inverse associations remained significant even after adjustment for traditional CVD risk factors including BMI, smoking, physical activity, alcohol consumption, history of diabetes, and intakes of energy, saturated, and unsaturated fats. The results did not change materially when the analyses were restricted only to dietary intake. A nonlinear dose-response meta-analysis demonstrated a linear inverse association in the analyses of folate and vitamin B6. A non-significant inverse association was found between dietary vitamin B12 intake and risk of CHD.

B-vitamins have several cardio-protective features that could explain these findings. Vitamin B6 has anti-inflammatory properties (Lotto, Choi, and Friso 2011), helps antioxidant defense system against oxidative stress (Hsu et al. 2015), and has some important functions in immune system (Rall and Meydani 1993). Folic acid can increase the generation of nitric oxide (Li and Forstermann 2009), has beneficial effects on endothelial function (Doshi et al. 2002), may be inversely associated with incident hypertension (Forman et al. 2005), and has possible antioxidant activity (Joshi et al. 2001). Also, B-vitamins are involved in one-carbon unit's metabolisms, and thereby, play important roles in DNA methylation and repair (Wasson et al. 2006; Zhong et al. 2017); mechanisms that have been shown play important roles for preventing chronic diseases.

In addition, several meta-analyses of interventional studies have presented strong evidence that supplementation with B-vitamins can decrease blood homocysteine concentrations (Clarke et al. 2010; HLTC 1998; Huang et al. 2012). However, despite homocysteine-lowering effects, B-vitamins supplementations did not decrease the risk of CHD(Bazzano et al. 2006; Clarke et al. 2010; Li et al. 2016; Miller et al., 2010). Therefore, interventional studies do not support B-vitamins supplementations for preventing CHD. Some possible explanations such as short term follow-up durations and high-dose supplementations with B-vitamins have been proposed to explain the null findings in interventional studies (Ntaios et al. 2009). In the present meta-analysis, in the analyses of folate and vitamin B6, a subgroup analysis by follow-up duration exhibited a non-significant association in the subgroup with shorter follow-up durations ($\leq$10 years *vs.* >10 years), which may confirm that the null association in interventional studies may be, in part, due to the relatively short term follow-up durations. Also, interventional studies generally include high-risk individuals. We found considerable stronger inverse associations in the subgroups of studies that excluded participants with preexisting CVD, which may show that B-vitamins may be useful for primary prevention of CHD, and not for secondary prevention.

In addition, interventional studies generally use high-dose supplementations which may be associated with a higher risk (Ntaios et al. 2009). In the present meta-analysis of prospective observational studies, we showed that increasing the intake folate and vitamin B6, within the usual daily intake ranges, were associated with a lower risk of CHD. This finding is in accordance with those of observational studies which have indicated that higher folate intake, within the usual daily intake range, was associated with lower homocysteine concentrations(Alfthan et al. 2003; Tucker et al. 1996). Also, an ecological analysis in Spain showed that higher B-vitamins intake was associated with a lower CHD mortality risk (Medrano et al. 2000).

The present review have several strengths. With the use of high-quality large-scale prospective cohort studies, we showed an inverse association between intake of folate and vitamin B6 and risk of CHD, which remained significant even after adjustment for major traditional CVD risk factors. Also, we conducted several subgroup and sensitivity analyses. We also were faced with some important limitations. Our main limitation may be the observational nature of primary studies. Interventional studies have reported null findings in this regard. Thus, it is difficult to conclude a causal relationship from the present results. However, in the subgroup analyses, we found non-significant associations in the subgroups of studies with shorter follow-up durations, as well as among studies without exclusion of participants with preexisting CVD. These findings show that B-vitamins intake may have protective effects against CHD in long-term, and not in short-term; as well as for primary prevention of CHD, and not for secondary prevention of CHD. However, the number of studies was low in the subgroup analyses, and further observational studies are needed to evaluate the associations. Second, higher intake of B-vitamins, especially folate, is associated with higher intake of other healthy foods such as fruit, vegetables, whole grains, and fibers. Of the 11 studies included in the present work, only three studies controlled for fiber intake, one study controlled for fish intake, and one study controlled for intake of vitamin C, vitamin E, and β-carotene. Thus, considering the inadequate adjustments for these confounding variables, we may have reached a biased conclusion. Third, the primary studies generally used a food frequency questionnaire to assess dietary intake, which overestimates the intake of healthy foods such as fruit and vegetables, and, as a result, water-soluble vitamins. Fourth, publication bias tests were performed with <10 studies, and therefore, their results may be due to the chance. Finally, the results were accompanied by moderate heterogeneity in the analysis of folate, and high heterogeneity in the analysis of B6, which may attenuate our final conclusion. However, only one study in the analysis of B6, and two studies in the analysis of folate, reported effect sized greater than one. Thus, the observed heterogeneity may be largely attributable to differences in the effect sizes of the studies examined, rather than inconsistencies in the direction of the association.

In summary, the present meta-analysis of prospective observational studies shows that higher intake of folate and vitamin B6, but not vitamin B12, is associated with a lower risk of CHD. Further observational studies are needed to evaluate the associations along with considering other dietary variables.

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