**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**

*Supplementary Materials including two supplementary tables, five supplementary figure, and MOOSE checklist*

**Supplementary Table 1**.Reported risk estimates in the primary studies in meta-analysis of B-vitamins and risk of coronary heart disease.

|  |  |  |  |
| --- | --- | --- | --- |
| **Vitamins intake categories**  | **Number of cases/ participants (personyears)** | **Relative risk (95%CI)** | **Covariates**  |
| Cui, 2010Folate, Men (µg/d) |
| < 272 | 54/4623 | 1.00 | Age, BMI, history of HTN and DM, smoking status, ethanol and energy intakes, as well as intakes of saturated fatty acids and n-3 and n-6 polyunsaturated fatty acids. |
| 272-351 | 43/4624 | 0.79 (0.52-1.19) |
| 352-430 | 39/4624 | 0.69 (0.45-1.07) |
| 431-535 | 52/4624 | 0.92 (0.60-1.40) |
| ≥536 | 45/4624 | 0.72 (0.45-1.14) |
| Cui, 2010Folate, Women (µg/d) |
| < 272 | 54/7122 | 1.00 | Age, BMI, history of HTN and DM, smoking status, ethanol and energy intakes, as well as intakes of saturated fatty acids and n-3 and n-6 polyunsaturated fatty acids. |
| 272-351 | 44/7122 | 0.97 (0.64-1.46) |
| 352-430 | 21/7122 | 0.43 (0.26-0.73) |
| 431-535 | 44/7122 | 0.89 (0.58-1.37) |
| ≥536 | 28/7122 | 0.57 (0.34-0.96) |
| Cui, 2010B6, Men (mg/d) |
| <0.79 | 58/4623 | 1.00 | Age, BMI, history of HTN and DM, smoking status, ethanol and energy intakes, as well as intakes of saturated fatty acids and n-3 and n-6 polyunsaturated fatty acids. |
| 0.79-0.96 | 46/4624 | 0.65 (0.41-1.01) |
| 0.97-1.11) | 41/4624 | 0.52 (0.30-0.88) |
| 1.12-1.32 | 42/4624 | 0.54 (0.29-0.98) |
| ≥1.33 | 46/4624 | 0.64 (0.32-1.30) |
| Cui, 2010B6, Women (mg/d) |
| <0.79 | 66/7122 | 1.00 | Age, BMI, history of HTN and DM, smoking status, ethanol and energy intakes, as well as intakes of saturated fatty acids and n-3 and n-6 polyunsaturated fatty acids. |
| 0.79-0.96 | 32/7122 | 0.48 (0.29-0.77) |
| 0.97-1.11) | 37/7122 | 0.55 (0.32-0.94) |
| 1.12-1.32 | 26/7122 | 0.40 (0.20-0.77) |
| ≥1.33 | 30/7122 | 0.47 (0.21-1.04) |
| Cui, 2010B12, Men (µg/d) |
| <4.5 | 50/4623 | 1.00 | Age, BMI, history of HTN and DM, smoking status, ethanol and energy intakes, as well as intakes of saturated fatty acids and n-3 and n-6 polyunsaturated fatty acids. |
| 4.5-5.9 | 50/4624 | 0.93 (0.58-1.49) |
| 6.0-7.6 | 44/4624 | 0.81 (0.46-1.43) |
| 7.7-9.8 | 47/4624 | 0.90 (0.49-1.67) |
| ≥9.9 | 42/4624 | 0.93 (0.48-1.80) |
| Cui, 2010B12, Women (µg/d) |
| <4.5 | 52/7122 | 1.00 | Age, BMI, history of HTN and DM, smoking status, ethanol and energy intakes, as well as intakes of saturated fatty acids and n-3 and n-6 polyunsaturated fatty acids. |
| 4.5-5.9 | 38/7122 | 0.97 (0.59-1.61) |
| 6.0-7.6 | 42/7122 | 1.71 (0.94-3.11) |
| 7.7-9.8 | 28/7122 | 1.18 (0.60-2.35) |
| ≥9.9 | 31/7122 | 1.39 (0.67-2.89) |
| Dalmeijer, 2008Folate (µg/d) |
| ≤169 | 131/Q1 | 1.00 | Age, HTN, cholesterolemia, mean SBP, total physical activity, BMI , smoking, DM, intake of energy, proteins (energy-adjusted), saturated fats (energy-adjusted), monounsaturated fats, polyunsaturated fats (energy-adjusted), alcohol (energy-adjusted), vitamin B2 (energy-adjusted) vitamin B6 (energy-adjusted), vitamin B12 (energy-adjusted), betaine (energy-adjusted) and choline (energy-adjusted). |
| 169 to ≤191 | 105/Q2 | 0.83 (0.61-1.12) |
| 191 to ≤215 | 125/Q3 | 0.99 (0.69-1.43) |
| > 215 | 132/Q4 | 1.05 (0.62-1.79) |
| Drogan, 2006Folate (µg/d) |
| <103 (median) | 69/11,123 | 1.00 | Age, sex, total energy intake, BMI, smoking history, alcohol consumption, regular exercise, educational attainment, history of DM, history of HTN and history of hyperlipidemia, daily intake of fiber, saturated fatty acids and polyunsaturated fatty acids. |
| ≥103 | 60/11,122 | 0.57 (0.36-0.91) |
| Van Guelpen, 2009Folate (µg/d) |
| <191 | 67/251 | 1.00 | BMI, ApoB ⁄ ApoA-I ratio, plasma cystatin C concentrations, current smoking, DM, HTN, education, and total homocysteine concentration. |
| 191-249 | 67/251 | 1.07 (0.64-1.78) |
| 249-328 | 62/251 | 0.89 (0.51-1.52) |
| >328 | 51/251 | 0.85 (0.47-1.51) |
| Van Guelpen, 2009B6 (mg/d) |
| <1.6 | 64/250 | 1.00 | BMI, ApoB ⁄ ApoA-I ratio, plasma cystatin C concentrations, current smoking, DM, HTN, education, and total homocysteine concentration. |
| 1.6-2.1 | 62/252 | 0.86 (0.50-1.46) |
| 2.1-2.8 | 77/251 | 1.18 (0.68-2.03) |
| >2.8 | 44/251 | 0.73 (0.34-1.34) |
| Van Guelpen, 2009B12 (µg/d) |
| <4.3 | 54/250 | 1.00 | BMI, ApoB ⁄ ApoA-I ratio, plasma cystatin C concentrations, current smoking, DM, HTN, education, and total homocysteine concentration. |
| 4.3-5.8 | 63/251 | 1.50 (0.86-2.62) |
| 5.8-7.6 | 51/252 | 0.56 (0.31-0.99) |
| >7.6 | 79/251 | 1.48 (0.87-2.52) |
| Ishihara, 2008Folate (µg/d) |
| 290 (median) | 54/87,090 (PY) | 1.00 | Age, sex, cigarette smoking, drinking, BMI, history of DM, education level, HTN, sports at leisure time, public health center, energy intake, as well as dietary intake of fish, saturated fatty acids, n-3 polyunsaturated fatty acids and n-6 polyunsaturated fatty acids. |
| 331 | 51/86,570 | 0.84 (0.56-1.26) |
| 372 | 43/86,415 | 0.81 (0.54-1.21) |
| 404 | 30/85,671 | 0.96 (0.63-1.45) |
| 436 | 45/84,833 | 0.75 (0.49-1.17) |
| Ishihara, 2008Folate (mg/d) |
| 1.30 (median) | 58/86,026 | 1.00 | Age, sex, cigarette smoking, drinking, BMI, history of DM, education level, HTN, sports at leisure time, public health center, energy intake, as well as dietary intake of fish, saturated fatty acids, n-3 polyunsaturated fatty acids and n-6 polyunsaturated fatty acids. |
| 1.40 | 54/86,932 | 0.78 (0.54-1.14) |
| 1.50 | 52/86,694 | 0.79 (0.53-1.17) |
| 1.55 | 39/86,143 | 0.57 (0.36-0.90) |
| 1.60 | 30/84,785 | 0.60 (0.37-0.97) |
| Ishihara, 2008B12 (µg/d) |
| 6.5 (median) | 57/86,902 | 1.00 | Age, sex, cigarette smoking, drinking, BMI, history of DM, education level, HTN, sports at leisure time, public health center, energy intake, as well as dietary intake of fish, saturated fatty acids, n-3 polyunsaturated fatty acids and n-6 polyunsaturated fatty acids. |
| 7.3 | 51/86,729 | 1.26 (0.87-1.84) |
| 8.6 | 41/85,828 | 0.82 (0.54-1.26) |
| 9.9 | 45/85,868 | 0.84 (0.54-1.31) |
| 11.1 | 39/85,253 | 0.75 (0.46-1.20) |
| Luu, 2011Folate, White (µg/d) |
| 0-155 | Q1 | 1.30 (1.09-1.56) | Age, gender, BMI, HDL, total cholesterol, current smoking status, DM, caloric intake, and HTN. |
| 156-211 | Q2 | 1.14 (0.97-1.34) |
| 212-278 | Q3 | 1.02 (0.88-1.19) |
| ≥279 | Q4 | 1.00 |
| Luu, 2011Folate, Black (µg/d) |
| 0-155 | Q1 | 1.36 (0.99-1.88) | Age, gender, BMI, HDL, total cholesterol, current smoking status, DM, caloric intake, and HTN. |
| 156-211 | Q2 | 1.27 (0.94-1.71) |
| 212-278 | Q3 | 1.02 (0.77-1.37) |
| ≥279 | Q4 | 1.00 |
| Marniemi, 2005 |
| Folate (third vs. first tertile) | 130/755 | 1.35 (0.82-2.24) | Age, gender, smoking and functional capacity. |
| B6 (third vs. first tertile) | 130/755 | 1.61 (0.95-2.75) |
| B12 (third vs. first tertile) | 130/755 | 1.45 (0.93-2.27) |
| Rimm, 1998Folate (µg/d) |
| 158 (median) | 237/Q1 | 1.00 | Age, time period, BMI, smoking, menopausal status, aspirin, vitamin E supplements, physical activity, HTN, parental history of MI, before age 65 years, alcohol, and intake of fiber, alcohol, saturated, polyunsaturated, and trans fats.  |
| 217 | 197/Q2 | 0.86 (0.71-1.05) |
| 276 | 197/Q3 | 0.86 (0.70-1.06) |
| 393 | 168/Q4 | 0.78 (0.63-0.98) |
| 696 | 140/Q5 | 0.69 (0.55-0.87) |
| Rimm, 1998B6 (mg/d) |
| 1.1 (median) | 216/Q1 | 1.00 | Age, time period, BMI, smoking, menopausal status, aspirin, vitamin E supplements, physical activity, HTN, parental history of MI, before age 65 years, alcohol, and intake of fiber, alcohol, saturated, polyunsaturated, and trans fats. |
| 1.3 | 210/Q2 | 0.92 (0.76-1.12) |
| 1.7 | 207/Q3 | 0.86 (0.70-1.05) |
| 2.7 | 175/Q4 | 0.88 (0.76-1.10) |
| 4.6 | 131/Q5 | 0.67 (0.53-0.85) |
| Voutilainen, 2001Folate (µg/d) |
| <211 | Q1 | 1.00 | Age, examination years, total cholesterol, HDL, LDL, TG, urinary excretion of nicotine metabolites, maximal oxygen uptake in exercise test, BMI, SBP, CHD in family, DM, intake of vitamin C, vitamin E, β-carotene, fiber, and saturated fatty acids |
| 211-236 | Q2 | 0.76 (0.55-1.06) |
| 237-261 | Q3 | 0.64 (0.46-0.89) |
| 262-297 | Q4 | 0.53 (0.33-0.87) |
| >297 | Q5 | 0.45 (0.25-0.81) |
| Voutilainen, 2001B6  |
| Above vs. below mean | 199/1980 | 0.82 (0.60-1.11) | Age, examination years, total cholesterol, HDL, LDL, TG, urinary excretion of nicotine metabolites, maximal oxygen uptake in exercise test, BMI, SBP, CHD in family, DM, intake of vitamin C, vitamin E, β-carotene, fiber, and saturated fatty acids |
| Voutilainen, 2001B12 |
| Above vs. below mean | 199/1980 | 0.73 (0.54-0.97) | Age, examination years, total cholesterol, HDL, LDL, TG, urinary excretion of nicotine metabolites, maximal oxygen uptake in exercise test, BMI, SBP, CHD in family, DM, intake of vitamin C, vitamin E, β-carotene, fiber, and saturated fatty acids |
| Zhao, 2018B6, Men (mg/d) |
| 1.3 (median) | 110/118,973 PY | 1.00 | Age at baseline, energy intake, education, income, occupation, smoke, alcohol, BMI, waist-hip ratio, physical activity, history of DM, HTN, coronary heart disease and stroke, vitamin B supplements use. |
|  1.53 | 105/119,988 | 0.93 (0.71-1.22) |
| 1.69 | 71/120,600 | 0.71 (0.52-0.96) |
| 1.87 | 78/121,289 | 0.81 (0.60-1.09) |
| 2.20 | 58/123,925 | 0.66 (0.47-0.91) |
| Zhao, 2018B6, Women (mg/d) |
| 1.24 (median) | 132/229,890 | 1.00 | Age at baseline, energy intake, education, income, occupation, smoke, alcohol, BMI, waist-hip ratio, physical activity, history of DM, HTN, coronary heart disease and stroke, vitamin B supplements use, menopausal status, and hormone replacement therapy. |
| 1.45 | 98/232,273 | 0.85 (0.65-1.11) |
| 1.62 | 75/233,661 | 0.81 (0.61-1.08) |
| 1.79 | 65/234,814 | 0.83 (0.61-1.13) |
| 2.11 | 60/235,497 | 0.91 (0.66-1.24) |
| **Abbreviations**: BMI, body mass index; CHD, coronary heart disease; DM, diabetes mellitus; HDL, high-density lipoprotein; HTN, hypertension; LDL, low-density lipoprotein; MI, myocardial infarction; SBP, systolic blood pressure; TG, triglycerides; PY, personyears; Q, quantile.  |

**Supplementary Table 2.** Subgroup analyses of vitamin B6 intake and risk of coronary heart disease (high *vs.* low analysis).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | n | RR (95%CI) | *I*2(%), *P*heterogeneity1 | *P*between2 |
| All studies |  | 8 | 0.81 (0.70-0.93) | 70%, 0.001 | - |
| Folate source |  |  |  |  |  |
|  Foods only |  | 6 | 0.86 (0.74-0.98) | 66%, 0.01 | 0.001 |
|  Foods and supplements |  | 3 | 0.68 (0.55-0.85) | 0%, 0.87 |
| Sex |  |  |  |  |  |
|  Men |  | 3 | 0.89 (0.83-0.96) | 0%, 0.52 | 0.29 |
|  Women |  | 3 | 0.81 (0.57-1.06) | 83%, 0.002 |
|  Both |  | 3 | 0.82 (0.43-1.21) | 55%, 0.11 |
| Geographical region |  |  |  |  |  |
|  US |  | 1 | 0.67 (0.51-0.83) | - | 0.01 |
|  Europe  |  | 3 | 0.89 (0.58-1.19) | 31%, 0.23 |
|  Asia |  | 4 | 0.85 (0.72-0.97) | 75%, 0.007 |
| Outcome  |  |  |  |  |  |
|  CHD incidence |  | 7 | 0.84 (0.73-0.95) | 68%, 0.005 | 0.03 |
|  CHD death |  | 1 | 0.54 (0.22-0.86) | - |
| Follow-up duration  |  |  |  |  |  |
|  ≤ 10 years |  | 2 | 1.10 (0.36-1.84) | 62%, 0.10 | 0.97 |
|  > 10 years |  | 6 | 0.80 (0.68-0.92) | 76%, 0.001 |
| Number of participants |  |  |  |  |  |
|  < 20,000 |  | 3 | 0.89 (0.58-1.19) | 31%, 0.23 | 0.74 |
|  > 20,000 |  | 5 | 0.80 (0.67-0.93) | 80%, < 0.001 |
| Exclusion preexisting CVD | Yes  | 6 | 0.70 (0.56-0.83) | 19%, 0.29 | < 0.001 |
|  | No | 2 | 0.93 (0.87-1.00) | 51%, 0.15 |
| Adjustments |  |  |  |  |  |
| Body mass index | Yes  | 7 | 0.80 (0.69-0.92) | 72%, 0.002 | 0.12 |
|  | No | 1 | 1.61 (0.71-2.51) | - |
| Smoking | Yes  | 7 | 0.81 (0.69-0.93) | 74%, 0.001 | 0.62 |
|  | No | 1 | 0.82 (0.51-1.13) | - |
| Alcohol consumption | Yes  | 5 | 0.80 (0.67-0.93) | 80%, < 0.001 | 0.74 |
|  | No  | 3 | 0.89 (0.58-1.19) | 31%, 0.22 |
| Physical activity  | Yes  | 4 | 0.83 (0.71-0.96) | 81%, 0.001 | 0.11 |
|  | No  | 4 | 0.78 (0.51-1.05) | 44%, 0.15 |
| History of diabetes | Yes  | 6 | 0.85 (0.74-0.96) | 61%, 0.02 | 0.01 |
|  | No  | 2 | 1.03 (0.14-1.93) | 75%, 0.04 |
| Energy intake  | Yes  | 5 | 0.80 (0.67-0.93) | 80%, < 0.001 | 0.74 |
|  | No  | 3 | 0.89 (0.58-1.19) | 31%, 0.23 |
| Intake of saturated fats | Yes  | 4 | 0.66 (0.54-0.78) | 0%, 0.62 | < 0.001 |
|  | No  | 4 | 0.93 (0.86-1.01) | 38%, 0.18 |
| Intake of polyunsaturated fats | Yes  | 3 | 0.64 (0.51-0.77) | 0%, 0.75 | < 0.001 |
|  | No  | 5 | 0.93 (0.86-1.00) | 25%, 0.25 |
| 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.  |



**Supplementary Figure 1**. Funnel plot of the relative risks of nine studies on folate intake and risk of coronary heart disease. Begg’s test *P*=0.25, Egger’s test *P*=0.72. Log RR: natural logarithm of relative risk. S.E: standard error.



**Supplementary Figure 2.** Relative risk of coronary heart disease for a 250 µg/d increment in folate intake. RR: relative risk.



**Supplementary Figure 3**. Funnel plot of the relative risks of eight studies on vitamin B6 intake and risk of coronary heart disease. Begg’s test *P*=0.71, Egger’s test *P*=0.27. Log RR: natural logarithm of relative risk. S.E: standard error.



**Supplementary Figure 4.** Relative risk of coronary heart disease for a 0.5 mg/d increment in vitamin B6 intake. RR: relative risk. SMHS: Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study.



**Supplementary Figure 5.** Relative risk of coronary heart disease for a 3 µg/d increment in vitamin B12 intake. RR: relative risk.

**MOOSE Checklist**

|  |  |  |
| --- | --- | --- |
| **Criteria**  | **Description** | **Pages** |
| **Reporting of background should include** |  |  |
| √ | Problem definition | See introduction | P3/4 |
| √ | Hypothesis statement | See introduction | P3/4 |
| √ | Description of study outcomes | Coronary heart disease | P5 |
| √ | Type of exposure or intervention used | Intakes of vitamin B6, B12, and folate  | P5 |
| √ | Type of study designs used | We included prospective cohort studies | P5 |
| √ | Study population | General population aged more than 18 years | P5 |
| **Reporting of search strategy should include** |  |  |
| √ | Qualifications of searchers | The credentials of the three investigators are indicated in the author gage. | Title page |
| √ | Search strategy, including time period included in the synthesis and keywords | PubMed and Scopus from their inception up to April 30, 2018 | P5 |
| √ | Databases and registries searched | PubMed and Scopus from inception up to April 30, 2018 | P5 |
| √ | Search software used, name and version, including special features | We did not employ search software. EndNote was used to merge retrieved citations and eliminate duplications | - |
| √ | Use of hand searching | We hand-searched bibliographies of retrieved papers for additional references, | P5 |
| √ | List of citations located and those excluded, including justifications | Details of the literature search process are outlined in the flow chart.  | P7/8 |
| √ | Method of addressing articles published in languages other than English | We placed restrictions on language to English papers  | P5 |
| √ | Method of handling abstracts and unpublished studies | We did not find relevant abstracts.  | - |
| √ | Description of any contact with authors | None. | - |
| **Reporting of methods should include** |  |  |
| √ | Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested | Detailed inclusion and exclusion criteria were described in the methods section.  | P5/Figure 1 |
| √ | Rationale for the selection and coding of data | Data extracted from each of the studies were relevant to the population characteristics, study design, exposure, outcomes, and possible effect modifiers of the association. | P6 |
| √ | Assessment of confounding | When a study provided several estimates with adjustment for different confounders, results were reported for the one adjusting for the largest number of factors.Subgroup analyses based on some of the study and participants characteristics and covariates were conducted  | P6 |
| √ | Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results | Each study was independently reviewed by two authors.  | P6/ Table 1 |
| √ | Assessment of heterogeneity | Heterogeneity of the studies were explored using Cochrane’s Q test of heterogeneity and I2 statistic that provides the relative amount of variance of the summary effect due to the between-study heterogeneity. | P6/7 |
| √ | Description of statistical methods in sufficient detail to be replicated | Descriptions of two types of analyses are detailed in Methods section. | P6/7 |
| √ | Provision of appropriate tables and graphics | We included 1 studies characteristics table, 1 summary results table, and four forest plots.  | Figures and Tables |
| **Reporting of results should include** |  |  |
| √ | Graph summarizing individual study estimates and overall estimate | Figure 2-4 | Figures |
| √ | Table giving descriptive information for each study included | Table 1 | Table 1 |
| √ | Results of sensitivity testing | See results | P9/10/11/12 |
| √ | Indication of statistical uncertainty of findings | 95% confidence intervals were presented with all summary estimates, I2 values and results of sensitivity analyses | P9/10/11/12 |
| **Reporting of discussion should include** |  |  |
| √ | Quantitative assessment of bias |  | P9/10/11/12 |
| √ | Justification for exclusion | We excluded studies that were outliers. | P9/10/11/12 |
| √ | Assessment of quality of included studies | We discussed the results of the sensitivity and subgroup analyses | P9/10/11/12 |
| **Reporting of conclusions should include** |  |  |
| √ | Consideration of alternative explanations for observed results |  | P12/13/14/15 |
| √ | Generalization of the conclusions |  | P16 |
| √ | Guidelines for future research |  | P16 |
| √ | Disclosure of funding source | This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. | P16 |