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A dose–response meta-analysis reveals an association between vitamin B_{12} and colorectal cancer risk

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Abstract

Objective: The current meta-analysis evaluated the association between vitamin B_{12} intake and blood vitamin B_{12} level and colorectal cancer (CRC) risk.
Design: The PubMed and EMBASE databases were searched. A dose–response analysis was performed with generalized least squares regression, with the relative risk (RR) and 95 % CI as effect values.
Setting: The meta-analysis included seventeen studies.
Subjects: A total of 10 601 patients.
Results: The non-linear dose–response relationship between total vitamin B_{12} intake and CRC risk was insignificant ($P = 0{\cdot}690$), but the relationship between dietary vitamin B_{12} intake and CRC risk was significant ($P < 0{\cdot}001$). Every 4·5 μg/d increment in total and dietary vitamin B_{12} intake was inversely associated with CRC risk (total intake: RR = 0·963; 95 % CI 0·928, 0·999; dietary intake: RR = 0·914; 95 % CI 0·856, 0·977). The inverse association between vitamin B_{12} intake and CRC risk was also significant when vitamin B_{12} intake was over a dosage threshold, enhancing the non-linear relationship. The non-linear dose–response relationship between blood vitamin B_{12} level and CRC risk was insignificant ($P = 0{\cdot}219$). There was an insignificant association between every 150 pmol/l increment in blood vitamin B_{12} level and CRC risk (RR = 1·023; 95 % CI 0·881, 1·187).
Conclusions: Our meta-analysis indicates that evidence supports the use of vitamin B_{12} for cancer prevention, especially among populations with high-dose vitamin B_{12} intake, and that the association between CRC risk and total vitamin B_{12} intake is stronger than between CRC risk and dietary vitamin B_{12} intake only.

Keywords
Vitamin B_{12}
Colorectal cancer
Meta-analysis

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide[1]. The development of CRC in the normal colorectal epithelium results from genetic alterations, epigenetic modifications and environmental factors[2–4]. Diet may be an important aetiological factor in CRC[5] and several biological mechanisms may explain the relationship between diet and CRC risk[5].

Vitamin B_{12} is an essential coenzyme for methionine synthase, which maintains adequate intracellular methionine levels in one-carbon metabolism[6,7] for several intracellular biological processes, including methylation reactions, nucleotide biosynthesis and DNA repair[8]. Recent studies have shown that one-carbon metabolism may be related to colorectal carcinogenesis[9,10]. Therefore, vitamin B_{12} deficiency may increase CRC risk[11,12]. However, several studies have reported that vitamin B_{12} is not associated with the risk of CRC[13,14], or that folic acid plus vitamin B_{12} is associated with increased cancer risk[15].

The results on the association between vitamin B_{12} intake and CRC risk are still controversial and no relevant pooled analyses have been performed. Therefore the aim of our meta-analysis study was to quantitatively and comprehensively summarize whether vitamin B_{12} intake or blood vitamin B_{12} level is related to CRC risk.

Methods

Literature search

A literature search for relevant studies was performed using the PubMed and EMBASE databases up to April 2014.

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The search terms used were: (vitamin B_{12} OR cyanocobalamin OR cobalamins OR cobalamin OR hydroxocobalamin OR 5′-deoxyadenosyl cobalamin) AND (colorectal cancer OR colon cancer OR rectal cancer). Moreover, we manually screened the references of the relevant studies and reviews to check for other potentially relevant studies.

Eligibility criteria

The studies which met the following eligible criteria were included: (i) cohort study or case–control study; (ii) the exposure of interest was vitamin B_{12} intake, or blood vitamin B_{12} level, for three or more quantitative categorized levels; (iii) the outcome of interest was related to CRC; and (iv) the risk estimates (OR, risk ratio (RR) or hazard ratio (HR)) and corresponding 95 % CI were reported or calculated from published data with a category-specific number of cases and a category-specific number of either person-years or non-cases. When several studies were based on the same population, only the most informative study was included.

Data extraction and quality assessment

Data were independently extracted by two reviewers. For each study, the following data were extracted: first author, publication year, publication country, study design, study name, population characteristics (sex and age), follow-up period, sample size, type of vitamin B_{12} intake (total intake = dietary intake plus dietary supplements; dietary intake; supplemental intake), measures and ranges of exposure, adjusted variables, and risk estimates with corresponding 95 % CI for each category. Risk estimates that reflected the greatest degree of adjustment for potential confounders were extracted.

The quality of the included studies was assessed according to the Newcastle–Ottawa Scale criteria[16]. A funnel plot was used to assess publication bias. Any disagreement on the data extraction and quality assessment of the studies was resolved through comprehensive discussion.

Statistical analysis

A dose–response analysis was first utilized to assess the relationship between vitamin B_{12} intake and blood vitamin B_{12} level and CRC risk using the generalized least squares method because this method can resolve the problem that the included studies used different FFQ[17,18]. The analytical method required the distribution of case and person-years, median values of vitamin B_{12} intake or blood vitamin B_{12} levels, and corresponding risk estimates in each category for each study. If there were results on both dietary and total B_{12} intake in one study, we used the results on total vitamin B_{12} intake for the main analyses. For each study, the mean or median value of vitamin B_{12} intake and blood vitamin B_{12} level in each category was assigned to each corresponding risk estimate. The assigned value of the lowest category was designated as a reference level. If the study did not provide mean or median values of exposure, the midpoint of the upper and lower boundaries in each category was assigned the median value of exposure[19]. For the open-ended exposure categories, the length of the open-ended interval was assumed to be the same as that of the adjacent interval[20].

We used random-effect restricted cubic splines with three knots at the 25 %, 50 % and 75 % percentiles of the distribution to examine a potential non-linear dose–response relationship between vitamin B_{12} intake and blood vitamin B_{12} level with CRC risk[21,22]. A P value for non-linearity was calculated by testing the null hypothesis that the regression coefficient of the second spline was equal to zero[21]. To test and verify the non-linear model, a meta-analysis comparing the appropriate open categories (or highest category) of exposure with the lowest category was performed. The non-linear dose–response relationship was also verified by several representative point values and the risk estimates of a subgroup analysis based on the range of exposure.

RR was used as a measure of the association between exposure and CRC risk. The OR provided by a case–control study was used as an estimate of the RR because the incidence of CRC was sufficiently rare[23]. When the category-specific risk estimates were reported separately for different polymorphisms in the 5,10-methylenetetrahydrofolate reductase gene, we combined the multiple risk estimates into a pooled estimate using a fixed-effects model for further meta-analysis[24]. When the RR of different population databases or population sex were presented in one study, we considered each population database as one study.

The heterogeneity of the studies was evaluated using the Cochran Q test and the I^2 statistic[25]. A P value <0·10 for the Q statistic and/or $I^2 > 50\,\%$ were considered to indicate statistically significant heterogeneity. The random-effects model was then used if there was significant heterogeneity; otherwise, the fixed-effects model was used. A subgroup analysis and a Galbraith plot were used to explore the sources of heterogeneity. Publication bias was evaluated with Egger's and Begg's tests[26,27], and a trim-and-fill analysis was conducted if publication bias was detected[28]. To explore the association between sample size and the RR for CRC, we constructed a sampling-based scatter plot graphically summarizing the association by modelling sample size as a continuous variable. A subgroup analysis was performed based on sample size. We also conducted subgroup analyses stratified by geographic region, type of vitamin B_{12} taken, range of exposure and tumour site.

A two-sided P value <0·05 was considered statistical significance. All statistical analyses were conducted using the statistical software package Stata version 12·0.

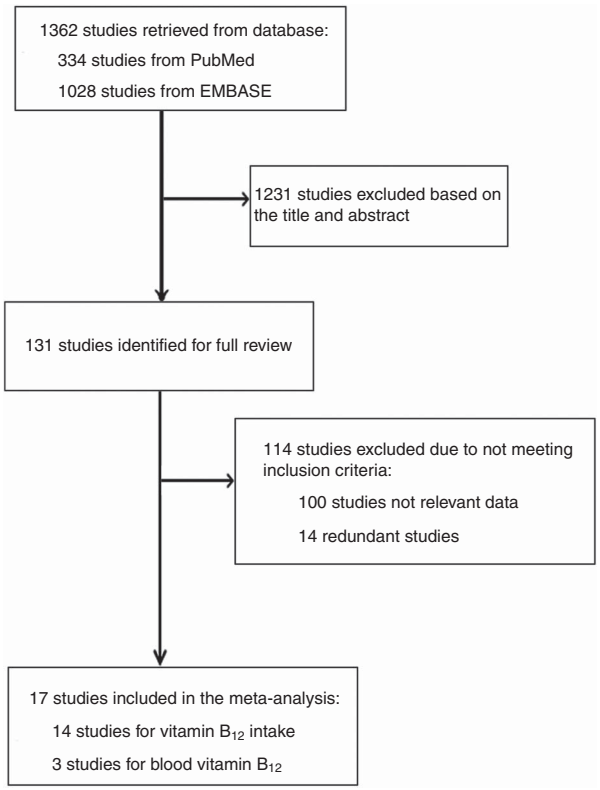

Fig. 1 Flowchart showing the literature search and study selection

Results

Selection of studies

Figure 1 shows a detailed flowchart of the literature search and the study inclusion procedure. A total of 1362 studies were initially identified with this literature search, but 1231 studies were excluded after screening the titles and abstracts. Then 114 studies were excluded after a full text review. Finally, fourteen studies of vitamin B_{12} intake and three studies of blood vitamin B_{12} level were identified as eligible for our meta-analysis.

Study characteristics

The fourteen studies of vitamin B_{12} intake included a total of 9693 cases (Table 1)[11–14,29–38]. The studies were conducted in Asia, Europe and North America, and were published between 2008 and 2013. In terms of the study design, five studies were cohort studies[13,29,31,34,36] and nine studies were case–control studies. Of these fourteen studies, eight studies provided results only for dietary vitamin B_{12} intake from food[11,30–34,37,38], three studies provided results only for total vitamin B_{12}[13,35,36], and three studies provided both total and dietary vitamin B_{12} intakes[12,14,29]. Besides the main end point of CRC, six studies provided sub-cohort results for colon cancer and/or rectal cancer separately[11,13,14,31,32,36]. Three case–control studies on blood vitamin B_{12} levels were conducted in the USA and Sweden, and were published between 2008 and 2010, comprising a total of 908 cases and 2387 controls (Table 2)[39–41].

Dose–response association between vitamin B_{12} intake and colorectal cancer risk

We first evaluated the non-linear dose–response relationship between vitamin B_{12} intake and CRC risk. Heterogeneity existed ($P_{\text{heterogeneity}} = 0{\cdot}017$) in the overall analysis of vitamin B_{12} intake, with a significant non-linear dose–response relationship ($P_{\text{non-linearity}} = 0{\cdot}026$; Fig. 2). In the non-linear dose–response relationship, there was a slight trend towards a reduction in CRC risk when vitamin B_{12} intake was less than 12·85 μg/d, although the reduction was not statistically significant, and a significantly reduced risk was observed when vitamin B_{12} intake was more than 12·85 μg/d. A slight reduction in CRC risk was also observed with every 4·5 μg/d increment in vitamin B_{12} intake (RR = 0·961; 95 % CI 0·930, 0·994) when we evaluated the relationship in a separate analysis with a linear model, suggesting that similar trends were observed with linear and non-linear models.

In addition, dose–response analyses for the association between vitamin B_{12} intake and CRC risk were stratified based on the type of vitamin B_{12} intake (total intake and dietary intake). There was no evidence of a non-linear association between total vitamin B_{12} intake and CRC risk ($P_{\text{non-linearity}} = 0{\cdot}690$), and every 4·5 μg/d increment in total vitamin B_{12} intake was inversely associated with CRC risk (RR = 0·963; 95 % CI 0·928, 0·999) without significant heterogeneity ($P_{\text{heterogeneity}} = 0{\cdot}138$). For dietary vitamin B_{12} intake there was significant evidence of non-linearity, and every 4·5 μg/d increment in dietary vitamin B_{12} intake was inversely associated with CRC risk (RR = 0·914; 95 % CI 0·856, 0·977) under the linear model.

The non-linear dose–response relationship (Fig. 2) graphically showed that vitamin B_{12} intake above a certain threshold (high dose, i.e. >12 μg/d) was inversely associated with CRC risk. We used several representative point values to test and verify the non-linear dose–response relationship by pooling the CRC risks for appropriate open categories of vitamin B_{12} intake when the lowest category in each study was regarded as a reference level. Our results indicated a significantly decreased risk of CRC when the vitamin B_{12} intake was above a certain threshold (8·5 μg/d: RR = 0·898; 95 % CI 0·824, 0·979; 11 μg/d: RR = 0·843; 95 % CI 0·731, 0·971; or 13 μg/d: RR = 0·881; 95 % CI 0·802, 0·968), thereby enhancing the non-linear dose–response relationship.

Dose–response association between blood vitamin B_{12} level and colorectal cancer risk

There was no heterogeneity ($P_{\text{heterogeneity}} = 0{\cdot}327$) in the overall analysis of blood vitamin B_{12} levels, with an insignificant non-linear dose–response relationship

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Table 1 The baseline characteristics of included studies on vitamin B_{12} intake and colorectal cancer

Article	Country	Study name	Study type	Age (years)	Cancer site	No. of participants (M/F)	No. of cases	Vitamin B_{12} type*	Exposure dose (μg/d)†	Study quality‡	Adjusted variables
Zschabitz *et al.* (2013)[29]	Germany	Women's Health Initiative Observational Study	Cohort	50–79	CRC	F: 86 820	808	Total	≤5·13 (Q1), >12·87 (Q4)	7	Age, BMI, race/ethnicity, past medical history of colonoscopy, smoking status, PA, postmenopausal hormone use
								Dietary	≤3·52 (Q1), >7·27 (Q4)		
Morita *et al.* (2013)[30]	Japan	Fukuoka Colorectal Cancer Study	CCS	20–74	CRC	M: 992 F: 639	816	Dietary	4·52 (Q1), 13·49 (Q5)	6	Sex, age, resident area, cigarette smoking, alcohol consumption, BMI, type of job, leisure-time PA, parental CRC, dietary intakes of Ca and *n*-3 PUFA
Bassett *et al.* (2013)[31]	Australia	Melbourne Collaborative Cohort Study	Cohort	27–80	CRC	M: 17 045 F: 24 469	910	Dietary	5·85 (Q5), 1·75 (Q1)	7	Country of birth, sex, education, alcohol consumption, PA, smoking status, family history of cancer, intake of cereal fibre
					CC	M: 17 045 F: 24 470	581				
					RC	M: 17 045 F: 24 471	326				
Liu *et al.* (2013)[32]	USA	Kaiser Permanente Medical Care Program	CCS	30–79	CC	M: 1944 F: 1639	982	Dietary	≤4·2 (T1), >6·9 (T3)	6	Age, sex, race/ethnicity, centre, BMI, lifetime vigorous activity, energy intake, dietary fibre, dietary Ca, cigarettes smoked, NSAID use, HRT. Participants with missing data for any of these variables were excluded
Key *et al.* (2012)[33]	UK	UK Dietary Cohort Consortium	CCS	Median: 62	CRC	M: 1246 F: 1270	565	Dietary	M: <3·34 (Q1), >6·42 (Q4) F: <2·46 (Q1), >5·03 (Q4)	6	Age, date of diary and sex, and adjusted for exact age, height, weight, energy intake, alcohol intake, fibre intake, smoking, education, social class, PA
Williams *et al.* (2010)[12]	USA	North Carolina Colon Cancer Study–Phase II	CCS	40–79	CRC: WA	M: 904 F: 616	720	Dietary	2·5 (Q1), 9·5 (Q4)	6	Age, sex, education, BMI, family history of CRC, NSAID use, total energy
					AA	M: 201 F: 183	225				
					WA	M: 904 F: 616	720	Total	3·3 (Q1), 13·5 (Q4)		
					AA	M: 201 F: 183	225				
Shrubsole *et al.* (2009)[34]	USA	Shanghai Women's Health Study	Cohort	40–70	CRC	F: 74 942	431	Dietary	1·28 (Q1), 4·86 (Q5)	5	Age, educational attainment, baseline household income, smoking status, drinking status, PA, HRT, menopausal status, family history of CRC, BMI, NSAID use, use of a B-vitamin supplement, history of colorectal polyps, diabetes history, daily intakes of energy, vegetables, fruits, red meats and Ca
Sharp *et al.* (2008)[35]	UK	Grampian Health Board	CCS	NR	CRC	M: 360 F: 312	264	Total	≤5·25 (Q1), ≥7·98 (Q4)	7	Sex, age, total energy, PA, family history of CRC, regular use of any NSAID, sex × NSAID interaction term; model for protein also adjusted for type of dietary supplement; model for alcohol also adjusted for type of dietary supplement and protein
Schernhammer *et al.* (2008)[36]	USA	Health Professionals Follow-up Study	Cohort	Median: 54	CC	M: 47 371	277	Total	≤6·0 (Q1), >16·1 (Q5)	6	Age, energy intake, sex, screening sigmoidoscopy, family history of CRC, aspirin use, smoking, PA in MET, BMI, history of colon polyps, beef intake, Ca intake, multivitamin use, and baseline folate, vitamin B_6, B_{12}, methionine and alcohol if not primary exposure
		Nurses' Health Study		Median: 47		F: 88 691	389		≤4·0 (Q1), >11·1 (Q5)		
Murtaugh *et al.* (2007)[14]	USA	Kaiser Permanente Medical Care Program	CCS	30–79	RC	M: 1000 F: 730	941	Total	≤6·09 (T1), >11·17 (T3)	7	Age, sex BMI, activity, energy, fibre, Ca, ibuprofen use, smoking (pack-years)
								Dietary	≤3·92 (T1), >6·57 (T3)		

Table 1 *Continued*

Article	Country	Study name	Study type	Age (years)	Cancer site	No. of participants (M/F)	No. of cases	Vitamin B_{12} type*	Exposure dose (μg/d)†	Study quality‡	Adjusted variables
Kune and Watson (2006)[11]	Australia	Melbourne Colorectal Cancer Study	CCS	NR	CRC CC RC	1442 1119 1050	715 392 323	Dietary	<4·1 (Q1), >11·1 (Q5)	5	Age, sex, alcohol, BMI, energy intake, family history of CRC, oral contraceptive pill use, cigarette pack-years, aspirin use, non-aspirin NSAID use
Otani *et al.* (2005)[38]	Japan	NR	CCS	20–74	CRC	M: 207 F: 124	107	Dietary	≤7·3 (T1), >11·2 (T3)	7	Smoking, alcohol consumption, BMI, total dietary fibre intake
Le Marchand *et al.* (2005)[37]	USA	Multiethnic Cohort	CCS	45–75	CRC	M: 1605 F: 1238	822	Dietary	≤2·86 (T1), >4·99 (T3)	5	Age at blood draw, sex, race/ethnicity
Harnack *et al.* (2002)[13]	USA	Iowa Women's Health Study	Cohort	55–69	CC RC	F: 35 216	598 123	Total	<5·13 (Q1), >18·35 (Q5) <7·18 (T1), >14·66 (T3)	6	Age, pack-years of cigarettes, BMI, oestrogen use, intakes of Ca, vitamin E and energy

M, males; F, females; CCS, case–control study; NR, not reported; CRC, colorectal cancer; CC, colon cancer; RC, rectal cancer; WA, white Americans; AA, African Americans; Q, quartile/quintile; T, tertile; PA, physical activity; NSAID, non-steroidal anti-inflammatory drug; HRT, hormone replacement therapy; MET, metabolic equivalent of task.
*Dietary vitamin B_{12} intake included vitamin B_{12} intake from foods only, and total vitamin B_{12} intake included vitamin B_{12} intake from foods and supplements.
†Exposure dose means the cut-off points or distribution for the highest and lowest categories of vitamin B_{12} intake.
‡The quality of the included studies was assessed with the nine-star Newcastle–Ottawa Scale criteria.

Table 2 The baseline characteristics of included studies on blood vitamin B_{12} level and colorectal cancer

Study	Country	Study name	Study type	Age (years)	Cancer site	No. of cases/controls	Exposure dose (pmol/l)*	Study quality†	Adjusted variables
Van Guelpen *et al.* (2010)[39]	Sweden	VIP Study, MONICA Study and MSP Study	CCS	NR	CRC: CIMP-low/high	91/181	M: ≤220 (Q1), >351 (Q4) F: ≤232 (Q1), >392 (Q4)	6	BMI, current smoking, recreational PA, alcohol intake
				62·5 (range 56·7–66·9)	CRC: CIMP-negative	92/183	M: ≤220 (Q1), >351 (Q4) F: ≤232 (Q1), >392 (Q4)		
Le Marchand *et al.* (2009)[40]	USA	Multiethnic Cohort	CCS	63–76	CRC	224/411	≤361 (Q1), >722 (Q4)	7	Age at blood draw, hours of fasting prior to blood draw, hours of moderate or vigorous PA, processed meat, pack-years, BMI, ethanol, family history of CRC, history of CRC, plasma folate
Weinstein *et al.* (2008)[41]	USA	ATBC Study	CCS	54–62	CRC	275/275	≤243 (Q1), >529 (Q5)	6	Age, BMI, occupational and leisure PA, intakes of vitamin D and Fe

VIP, Västerbotten Intervention Programme; MONICA, MONitoring of trends and determinants in CArdiovascular disease; MSP, Mammography Screening Project; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention; CCS, case–control study; NR, not reported; CRC, colorectal cancer; CIMP, CpG island methylator phenotype; M, males; F, females; Q, quartile/quintile; PA, physical activity.
*Exposure dose means the cut-off points or distribution for the highest and lowest categories of blood vitamin B_{12} level.
†The quality of the included studies was assessed with the nine-star Newcastle–Ottawa Scale criteria.

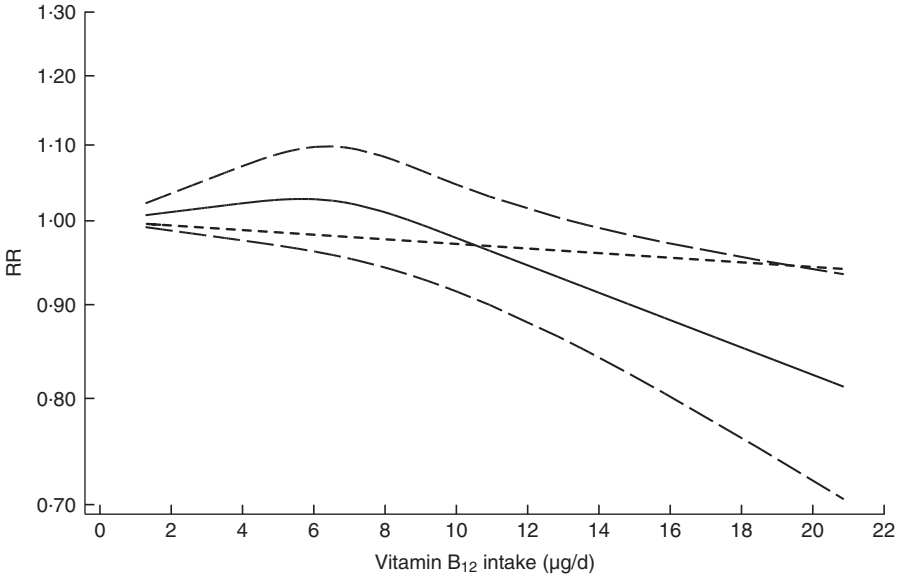

Fig. 2 Dose–response relationship between vitamin B_{12} intake and risk of colorectal cancer. Relative risks (RR; ———) and the corresponding 95 % CI (— — —) were summarized for the dose–response relationship between vitamin B_{12} intake (μg/d) and risk of colorectal cancer. Data were modelled with random-effects restricted cubic spline models, where - - - - represents the linear trend

between blood vitamin B_{12} level and CRC risk ($P_{\text{non-linearity}} = 0{\cdot}219$). The results of the linear model indicated that every 150 pmol/l increment in blood vitamin B_{12} level was not associated with CRC risk (RR = 1·023; 95 % CI 0·881, 1·187) without significant heterogeneity ($P_{\text{heterogeneity}} = 0{\cdot}303$).

High v. *low vitamin B_{12} intake or blood vitamin B_{12} level*

In the crude analyses, the highest vitamin B_{12} intake or blood vitamin B_{12} level *v.* the lowest intake or blood level was insignificantly associated with CRC risk (vitamin B_{12} intake: RR = 0·942; 95 % CI 0·829, 1·070; blood vitamin B_{12} level: RR = 0·927; 95 % CI 0·560, 1·534). We also performed in-depth subgroup analysis of the vitamin B_{12} intake. The results indicated that the association between vitamin B_{12} intake and CRC risk was stronger in studies with a wider range of vitamin B_{12} intake (>8 μg/d difference in assigned value compared with the reference level: RR = 0·831; 95 % CI 0·711, 0·971; Fig. 3) compared with studies with a narrower range of vitamin B_{12} intake (≤8 μg/d: RR = 1·016; 95 % CI 0·878, 1·175), thereby enhancing the non-linear dose–response relationship. The highest *v.* lowest total vitamin B_{12} intake was significantly associated with CRC risk (RR = 0·870; 95 % CI 0·782, 0·967).

The association between sample size and the RR of CRC is summarized in Fig. 4 with a sampling-based scatter plot. The subgroup analysis based on sample size showed that the sample sizes of the studies did not obviously influence our results. Detailed subgroup analyses based on CRC sites, sex of participants, regions of participants and the adjusted covariates were also conducted, and the results are summarized in Table 3.

Publication bias

Begg's test and Egger's test showed no evidence of publication bias for the overall analysis of vitamin B_{12} intake ($P_{\text{Begg}} = 0{\cdot}192$, $P_{\text{Egger}} = 0{\cdot}266$) and blood vitamin B_{12} level ($P_{\text{Begg}} = 1{\cdot}000$, $P_{\text{Egger}} = 0{\cdot}447$), and the subgroup analysis of total vitamin B_{12} intake ($P_{\text{Begg}} = 0{\cdot}348$, $P_{\text{Egger}} = 0{\cdot}616$).

Discussion

The one-carbon metabolism pathway requires adequate vitamin B_{12} and this raises the possibility that vitamin B_{12} may have an important role in CRC risk. However, the association between vitamin B_{12} and CRC risk is still under debate due to a lack of sufficient evidence. To the best of our knowledge, our present dose–response meta-analysis is the first study to systematically evaluate the association between vitamin B_{12} and CRC risk.

In crude overall analyses, vitamin B_{12} intake or blood vitamin B_{12} level was insignificantly associated with CRC risk. Interestingly, current results found a non-linear dose–response relationship between vitamin B_{12} intake and CRC risk. The association was insignificant if vitamin B_{12} intake was under a certain threshold (low dosage, i.e. <7 μg/d), whereas vitamin B_{12} intake above a certain threshold (high dosage, i.e. >12 μg/d) was inversely associated with CRC risk. Moreover, this non-linear dose–response relationship was enhanced by several representative point values and the risk estimates of subgroup analysis based on range of exposure (Fig. 3).

The biological mechanisms responsible for the protective effect of high-dosage vitamin B_{12} are unclear. One possible explanation is that vitamin B_{12} is an essential

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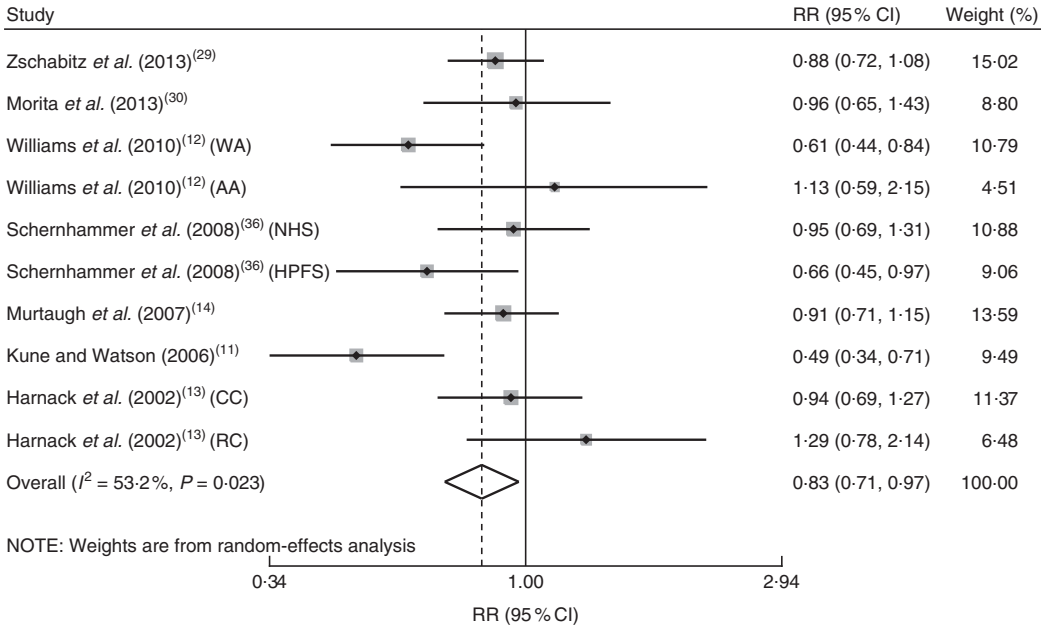

Fig. 3 Adjusted relative risk (RR) of colorectal cancer for a wider range of vitamin B_{12} intake (range >8 μg/d); the adjusted RR was summarized for the association between a wider range of vitamin B_{12} intake and risk of colorectal cancer. The study-specific RR and 95 % CI are represented by the black dot and horizontal line, respectively; the area of the grey square is proportional to the specific-study weight to the overall meta-analysis. The centre of the open diamond presents the pooled RR risk and its width represents the pooled 95 % CI (NHS, Nurses' Health Study; HPFS, Health Professionals Follow-up Study; CC, colon cancer; RC, rectal cancer)

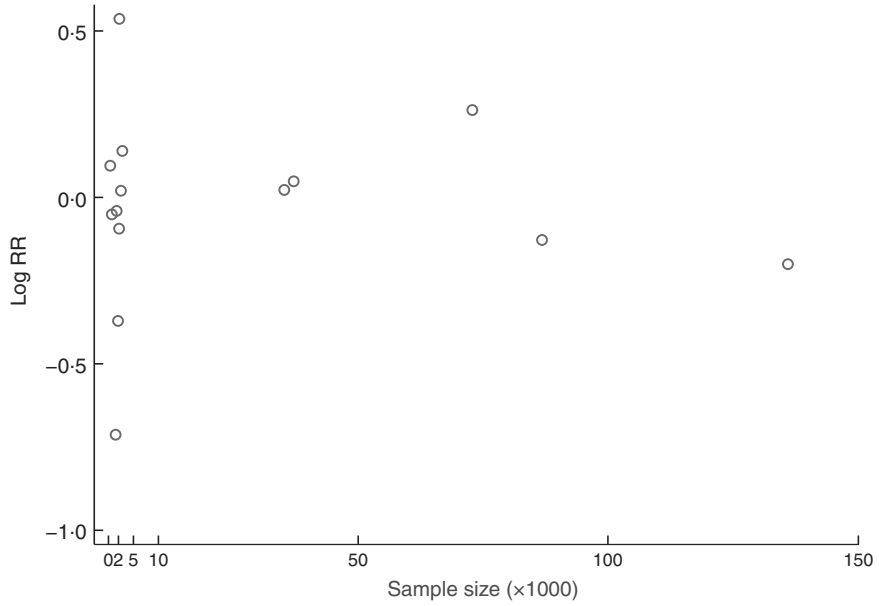

Fig. 4 The association between sample size and the relative risk (RR) of colorectal cancer; a sampling-based scatter plot summarized the association between sample size and the RR of colorectal cancer

coenzyme in one-carbon metabolism for methylation reactions, nucleotide biosynthesis and DNA repair through transforming homocysteine into methionine. Indeed, Choi *et al.* reported that the colonic DNA of vitamin B_{12}-deficient rats has a 35 % decrease in genomic methylation and a 105 % increase in uracil incorporation, which might increase susceptibility to carcinogenesis[42]. Furthermore, B-group vitamin supplementation, including vitamin B_{12}, may have antioxidant and anti-inflammatory effects[43,44]. In addition, animal experiments have shown that vitamin B_{12} can inhibit the proliferation of cancer cells[45].

We did not find statistically significant evidence for a non-linear relationship between blood vitamin B_{12} level and CRC risk ($P_{\text{non-linearity}} = 0{\cdot}219$). Every 150 pmol/l

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Table 3 The results of subgroup analyses for the relationship between vitamin B_{12} intake and colorectal cancer risk

Subgroups	No. of studies	No. of independent cohorts*	Effect model†	RR‡		P§	I^2 (%)‖
				Estimate	95 % CI		
All studies	14	17	Random	0·942	0·829, 1·070	0·357	55·60
Type of vitamin B_{12} intake							
Total	6	9	Fixed	0·870	0·782, 0·967	0·010	22·90
Food	11	12	Random	0·960	0·810, 1·137	0·635	67·00
Range of exposure							
≤8 μg/d	10	11	Random	1·016	0·878, 1·175	0·835	49·50
>8 μg/d	7	10	Random	0·831	0·711, 0·971	0·020	53·20
Study type							
Cohort	5	7	Fixed	0·962	0·862, 1·074	0·488	32·30
Case–control	9	10	Random	0·925	0·748, 1·144	0·472	66·00
Cancer type							
Colon	5	6	Random	0·867	0·655, 1·149	0·321	73·30
Rectal	4	4	Random	0·958	0·669, 1·372	0·815	70·20
Sample size							
≤3000	9	10	Random	0·925	0·748, 1·144	0·472	66·00
>3000	5	7	Fixed	0·962	0·862, 1·074	0·488	32·30
Sex							
Female	5	6	Fixed	0·995	0·872, 1·135	0·940	35·70
Regions of participants							
USA (all¶)	7	10	Random	0·939	0·802, 1·100	0·436	53·60
USA (total**)	5	8	Fixed	0·866	0·777, 0·966	0·010	31·80
USA (food††)	5	6	Random	0·979	0·761, 1·260	0·868	70·60
Europe (all)	4	4	Random	0·849	0·601, 1·198	0·352	77·00
Europe (total)	1	1	NA	0·950	0·560, 1·630	NA	NA
Europe (food)	3	3	Random	0·823	0·538, 1·261	0·371	84·70
Asia (all)	3	3	Fixed	1·123	0·872, 1·445	0·369	0·00
Asia (total)	NA	NA	NA	NA	–	NA	NA
Asia (food)	3	3	Fixed	1·123	0·872, 1·445	0·369	0·00
Adjustment for confounders							
Smoking							
Yes	11	13	Random	0·954	0·830, 1·097	0·511	56·50
No	3	4	Random	0·903	0·634, 1·286	0·572	61·60
Alcohol							
Yes	7	7	Random	0·943	0·747, 1·188	0·617	63·20
No	7	10	Random	0·939	0·802, 1·100	0·436	53·60
Physical activity							
Yes	8	9	Random	0·967	0·875, 1·069	0·517	39·60
No	6	8	Random	0·894	0·700, 1·141	0·368	67·00
Family history							
Yes	5	7	Random	0·806	0·614, 1·058	0·120	69·20
No	9	10	Random	1·002	0·911, 1·103	0·966	0·00
Vitamin B_{12} intake (μg/d)							
≥8	10	27	Random	0·919	0·842, 1·004	0·061	42·20
≥8·5	9	25	Random	0·898	0·824, 0·979	0·014	35·90
≥9	8	23	Random	0·896	0·816, 0·985	0·023	41·20
≥9·5	8	20	Random	0·901	0·812, 1·001	0·051	46·30
≥10	8	17	Random	0·890	0·793, 1·000	0·050	51·00
≥10·5	8	16	Random	0·889	0·786, 1·005	0·061	54·10
≥11	8	13	Random	0·843	0·731, 0·971	0·018	53·70
≥11·5	8	13	Random	0·843	0·731, 0·971	0·018	53·70
≥12	8	13	Random	0·843	0·731, 0·971	0·018	53·70
≥12·5	8	13	Random	0·843	0·731, 0·971	0·018	53·70
≥13	7	12	Fixed	0·881	0·802, 0·968	0·008	34·30
≥13·5	5	9	Random	0·858	0·736, 1·000	0·050	49·50

RR, relative risk; NA, not applicable.
*The actual number of 'independent study cohorts' that can be included in the corresponding analysis because we considered each population database as one study cohort for statistical analyses if the RR of different populations or population sex were available (one study); thus the number of independent study cohorts can be larger than the number of studies.
†Random-effects model was used if there was significant heterogeneity; otherwise, fixed-effects model was used.
‡RR for colorectal cancer risk of the highest *v.* lowest categories of vitamin B_{12} intake.
§P for the RR.
‖I^2 shows the degree of heterogeneity among studies.
¶'All' means that the analysis included either total vitamin B_{12} intake or dietary vitamin B_{12} intake.
**'Total' means that the analysis included only total vitamin B_{12} intake.
††'Food' means that the analysis included only dietary vitamin B_{12} intake.

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increment in blood vitamin B_{12} level was not associated with CRC risk, enhanced by the analysis of the highest blood vitamin B_{12} levels *v.* lowest blood levels. One explanation may be that all included studies roughly assessed blood vitamin B_{12} level in relation to CRC risk while ignoring the fact that only methylcobalamin and 5′-deoxyadenosylcobalamin are the principal active coenzyme forms of vitamin B_{12} compared with other forms of vitamin B_{12} in blood (e.g. cyanocobalamin and hydroxocobalamin)[46–48]. Thus, these inactive forms of blood vitamin B_{12} may underestimate the effect of active forms of vitamin B_{12} on CRC risk. Similar to studies regarding pyridoxal 5′-phosphate (PLP, the active form of vitamin B_6), the association between the active forms of vitamin B_{12} and CRC risk should be deeply investigated by further multicentre clinical studies.

Our meta-analysis indicated no association between blood vitamin B_{12} level and CRC risk, different from the association found between vitamin B_{12} intake and CRC risk. One potential reason may be that plasma vitamin B_{12} level has been found to be only slightly correlated with dietary vitamin B_{12} intake (multivariable Pearson correlation coefficient, $r=0{\cdot}08$) and total vitamin B_{12} intake ($r=0{\cdot}25$)[49].

The strength of the current meta-analysis study was that our results and conclusions were enhanced by an in-depth subgroup analysis. A subgroup analysis based on the type of vitamin B_{12} intake indicated that the association between total vitamin B_{12} and CRC risk was stronger than the association between dietary vitamin B_{12} intake alone and CRC risk (Table 3). As expected, heterogeneity was reduced to low levels in the assessment of total vitamin B_{12} intake. Supplemental vitamin B_{12} intake may play an important role in CRC risk, and Zschabitz *et al.* showed that supplemental vitamin B_{12} intake was marginally associated with CRC risk[29]. However, the interaction between supplemental vitamin B_{12} intake and dietary vitamin B_{12} intake is unclear. Thus, the type of vitamin B_{12} intake may be important for the assessment of vitamin B_{12} intake and CRC risk, and future clinical studies should not ignore the role of supplemental vitamin B_{12} intake. The association between vitamin B_{12} intake and CRC risk may be various among different races of population (Table 3), in agreement with the study by Williams *et al.* in which the associations between vitamin B_{12} and distal CRC differed for whites compared with African Americans[12]. This may be due to polymorphisms in genes related to one-carbon metabolism, supported by a previous study in which gene–nutrient interactions in folate-mediated one-carbon metabolism played an important role in modifying the risk of CRC[32]. No evidence of publication bias was detected, indicating that the entire pooled results may be unbiased.

Several limitations of our meta-analysis must be acknowledged. First, the controls were not always of uniform ascertainment in case–control studies, although most studies matched controls to cases based on age and sex. Some controls might therefore have had benign diseases or other risk factors that contribute to CRC risk and misclassifications of controls or vitamin B_{12} intake possibly exist among several included studies. Second, some important confounding factors were not measured in several studies and some inherently confounding factors in included studies could not be solved perfectly in our study. Thus, an inadequate control of confounding factors may lead to an underestimation of risk estimates of vitamin B_{12} on CRC. Third, the limited number of included studies may impact the statistical power and performance of some in-depth subgroup analysis. The assigned value of vitamin B_{12} in each category (midpoint, mean and median) was not available in all included studies for

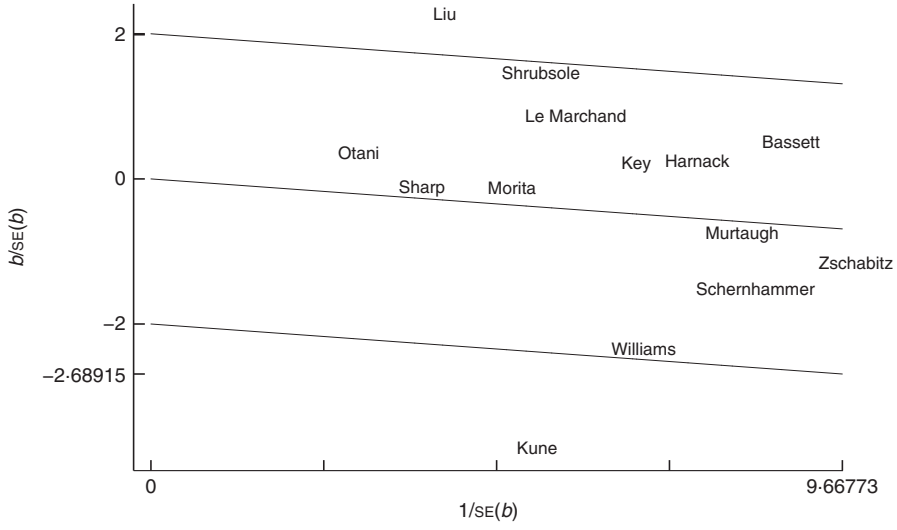

Fig. 5 Galbraith plot for exploring the sources of heterogeneity in the fourteen studies examining the relationship of vitamin B_{12} intake and risk of colorectal cancer. ——— represent fitted lines; those at ±2 from the fitted (regression-through-the-origin) line represent the approximate 95 % confidence region; the studies are denoted by the first author's surname

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dose–response analysis. Therefore, various assigned values might affect the accuracy of the dose–response relationship. In addition, a considerable degree of heterogeneity was observed among studies and could not be explained completely. Our subgroup analysis indicated that study design may have been one source of heterogeneity. After the case–control studies were excluded, the heterogeneity among the studies decreased ($I^2 = 32{\cdot}3\,\%$) and the fixed-effects model was used. Similarly, our results also showed that the types of vitamin B_{12} intake and population sex contributed to the heterogeneity (total vitamin B_{12} intake group: $I^2 = 22{\cdot}9\,\%$; female group: $I^2 = 35{\cdot}7\,\%$). For individual studies, the result of the Galbraith plot (Fig. 5) indicated that the studies by Liu *et al.*[32] and Kune and Watson[11] contributed substantial heterogeneity. Indeed, the heterogeneity was clearly reduced after the removal of one or both of these studies (without Liu *et al.*: $I^2 = 49{\cdot}5\,\%$; without Kune and Watson: $I^2 = 32{\cdot}7\,\%$; without Liu *et al.* and Kune and Watson: $I^2 = 9{\cdot}4\,\%$). The remaining unexplained heterogeneity may have been caused by differences in the population characteristics and methodological differences (e.g. differences in the estimates of vitamin B_{12} intake in FFQ and the control of confounding factors). The heterogeneity did not influence or dominate the quality or stability of the results. Despite these limitations, the present study is the first dose–response meta-analysis to quantitatively assess the association between vitamin B_{12} and CRC risk.

Conclusion

In conclusion, results from the present meta-analysis indicate that vitamin B_{12} intake is inversely associated with CRC risk when the dosage of vitamin B_{12} intake is above a certain threshold, and that the association between total vitamin B_{12} and CRC risk is stronger than the association between dietary vitamin B_{12} intake alone and CRC risk. In addition, there was an insignificant association between blood vitamin B_{12} level and CRC risk. Further studies are required to investigate the associations between the active forms of blood vitamin B_{12} and CRC risk and to evaluate the influence of vitamin B_{12} supplementation.

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