The association of homocysteine, folate, vitamin B12, and vitamin B6 with fracture incidence in older adults: a systematic review and meta-analysis

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Background: Diverse conclusions have been drawn regarding the association of homocysteine (HCY) deficiency and supplements of B vitamins with fracture incidence in older adults. The aim of this meta-analysis was to investigate the association of HCY and B vitamins (folate, vitamin B12, and B6) with fracture incidence in older adults and whether supplements of B vitamins reduce the risk of fracture.

Methods: The PubMed, Embase, and Cochrane library databases were systematically searched from their inception dates to 1 July 2019 to identify relevant published articles. Meta-analysis was performed to pool hazard ratios (HRs) or risk ratios (RRs) and 95% confidence intervals (CIs) using a random effects model.

Results: A total of 28 studies fulfilled the inclusion criteria. High serum HCY was an independent risk factor for fractures in older persons (HR =1.25, 95% CI: 1.12 to 1.40), but only at the highest quartile level (>15 μmol/L) (HR =1.71, 95% CI: 1.37 to 2.12), rather than the second and third quartile. Multiple sensitivity and subgroup analyses supported the consistency and stability of this result. A severe deficiency of folate, instead of vitamin B12 and B6, was found to increase the risk of fracture in older adults (HR =1.46, 95% CI: 1.06 to 2.02; 1.24, 95% CI: 0.79 to 1.95; 1.36, 95% CI: 0.90 to 2.06, respectively). For the interventional effect, there was no significant association of combined folate and vitamin B12, combined folate, vitamin B12 and B6, or single vitamin B6 supplementation with the decrease of fracture risk.

Discussion: This meta-analysis revealed that significantly elevated serum level of HCY is positively associated with fracture incidence in older adults, yet the necessity and threshold for intervention by B vitamins require further large-scale high-quality clinical trials to validate.

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Keywords: Fractures; homocysteine; B vitamins; meta-analysis; systematic reviews

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Introduction

The increased prevalence of aging-related fractures among people worldwide leads to a higher morbidity and mortality accompanied by a heavy economic burden on public health (1-3). Multiple factors, including deterioration of bone mineral density (BMD), low body mass index, malnutrition, neurological diseases, and metabolic disorders such as diabetes and homocystinuria contribute to the elevation of fracture incidence in older adults (4-8). Some risk factors such as low bone density, calcium deficiency and vitamin D deficiency have been well investigated. However, ageing-related metabolic products, as potential risk factors and promising interventional targets for fractures in older adults, were poor investigated but has drawn ever-increasing attention in the last two decades. Among them, the role of homocysteine (HCY), one of the most investigated metabolites in geriatric cardiovascular diseases, in aging-related fractures remains unclear.

The sulfur-containing amino acid, HCY, is produced during the metabolism of methionine. Numerous studies have shown that HCY is a risk factor of aging-related diseases, such as cardio-/cerebrovascular diseases, diabetes, and neural disease (9-13). In addition, accumulated evidence indicates that high serum homocysteine (HHCY) is associated with higher incidence of skeletal deformities, including osteoporosis, which is a key risk factor for osteoporotic fracture (5,14,15). Furthermore, the intervention of HCY using B vitamins has been reported to reduce the incidence of targeted aging-related diseases (10,11,16,17). Hence, we hypothesized that the risk of osteoporotic fractures, as a major aging-related problem, can also be reduced by supplementation of B vitamins.

Folate, vitamin B12, and vitamin B6, as members of the B vitamin group, are the 3 key enzymes in the metabolism of HCY that can be conveniently supplemented in the older community via nutrient intake (18). Evidence shows that supplementation of folate, vitamin B12, and/or vitamin B6 lower the level of serum HCY which in theory can subsequently reduce osteoporotic fracture incidence (15). However, mixed conclusions have been drawn regarding the association between the supplements of B vitamins with fracture incidence in older adults (15,19-21).

There is a range of factors influencing fracture incidence, among which the compromise of BMD plays one of the most key roles in increasing fracture risk in the older population. Accumulating evidence has stated a strong association of HHCY with a decrease of BMD (22-24). Recent studies have shown that HCY-lowering intervention can also improve BMD, indicating the causation of HCY and BMD (6,23,25-27). Furthermore, 1 study (25) analyzed cross-sectional data involving 6,100 participants from 3 large Dutch studies including the cross-sectional data of the B-PROOF (B-Vitamins for the Prevention of Osteoporotic Fractures) study and 2 cohorts of the Rotterdam Study (RS-I and RS-II), and confirmed this association.

The underlying mechanism may be its effect on osteoclasts and osteoblasts, but the natural mechanism remains unclear (28). Both *in vitro* and *in vivo* studies have indicated that HHCY can disrupt the process of collagen cross-linking in bone tissue and then cause compromised bone strength (29-32). Moreover, owing to HCY's role in cerebrovascular and neural diseases, it has emerged from increasing evidence that HCY can increase the risk of stroke and Parkinson's disease, harming the coordination function and subsequently raising the risk of falling and fracture incidence (11,33).

The aim of this meta-analysis was to investigate the association of HCY and B vitamins (folate, vitamin B12, and B6) with fracture incidence in older men and women and whether supplementation with B vitamins can reduce the risk of fracture.

We present the following article in accordance with the PRISMA reporting checklist (available at https://dx.doi.org/10.21037/atm-21-2514).

Methods

This meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-analyses statement (PROSPERO identifier: CRD42019122586).

Search trials

Published articles were retrieved utilizing two methods. First, 3 scientific databases (PubMed, Embase, and the Cochrane Library) were searched to identify published articles evaluating the association of HCY, deficiency and supplements of B vitamins (folate, vitamin B12, and B6) with the incidence of fracture using a combination of keywords and MeSH terms "homocysteine", "homocysteic acid", "HCY", "vitamin", "cobalamin", "cyanocobalamin", "B12", "pyridoxal", "pyridoxine", "pyridoxamine", "B6", "folate", "folic acid", "folacin", "B9" AND "fracture" OR "bone". Second, all reference lists of relevant articles

(reviews, systematic reviews, meta-analyses, and included studies) were further screened manually to retrieve additional studies that were not listed in the databases. The last search was updated on 1 August 2020 with no restrictions on language, date, or journal of publication.

Inclusion and exclusion criteria

Studies were selected based on the following inclusion criteria: (I) participants, mean age of people enrolled in the trials was older than 50 years; (II) interventions, high or low level of HCY, folate, vitamin B6, or B12; (III) control group, high or low level of HCY, folate, vitamin B6, or B12; (IV) outcome, trials evaluated the relationship of HCY, folate, vitamin B6, or B12 with the risk of fracture; and (V) study design, randomized or quasi-randomized controlled clinical trials, retrospective/prospective matched cohort studies.

The exclusion criteria were as follows: (I) comments, reviews, meta-analysis, editorials, and other non-original trials; (II) congress proceedings and abstracts; (III) animal experiments; and (IV) studies providing no data to calculate evaluation indexes [hazard ratio (HR) or risk ratio (RR)]. For articles containing overlapping data, those presenting the most comprehensive data or that were published the most recently were selected. We first removed redundant and unrelated records by reading titles and abstracts. Then the full texts of remainders were downloaded to confirm their eligibility based on above criteria.

Quality assessment

The methodological qualities of eligible studies were estimated utilizing two methods based on data type. First, for the data of dichotomous variables, the Newcastle-Ottawa Scale (NOS) with 9 factors considered was used. A score of 1 was given to a study for each item. The quality scale ranged from a score of 0 to 9 and studies with high scores were considered good reports. Studies with scores ≥7 were regarded as high-quality reports.

Second, a quality assessment tool based on Cochrane risk-of-bias criteria was used to evaluate the methodological quality of the included studies involving the data of randomized controlled trials (RCTs). This tool contains 7 items used to assess bias in each trial that included the randomization sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias, and each paper was described as low risk, high risk, or unclear risk.

Data extraction

To reduce potential bias, 2 investigators (A & B) conducted the process of data extraction blindly and independently. Any discrepancies were resolved by consultation with a third investigator. Each included study deemed appropriate for inclusion listed the first author's surname, publication year, journal, country of origin, participant characteristics (number, age, and gender), doses of folate, vitamin B6, or vitamin B12; baseline serum, follow-up, fracture type, statistical index, and adjustments factors of Cox-regression. If the studies had more than two groups or factorial designs and permitted multiple comparisons, the information and data that ruled out more factors was extracted. When those data were our outcomes of interest, we pooled them with the data from primary trials.

Statistical analysis

The correlation of folate, vitamin B6, vitamin B12, or HCY with fracture incidence was assessed by calculating the pooled HRs or RRs and their 95% confidence interval (CI). Based on the practice recommendation of the Cochrane Handbook, heterogeneity was assessed using the I-square (I^2) statistic. Meanwhile, an I^2<50% was considered not significant. When heterogeneity was significant, the potential sources of heterogeneity were identified by analyzing the methodological variability of the included studies. To reduce potential bias, a random-effects model was used for all subgroup meta-analyses. Additionally, sensitivity analysis was performed by omitting studies 1 by 1 to evaluate the impact of a single trial on the overall pooled estimate. Egger's test was used to evaluate the possibility of publication bias. The software STATA, version 12.0 (Stata Corp, College Station, TX, USA), was applied for all statistical analyses, and P<0.05 was considered statistically significant.

Results

Selection process

The detailed article search and study selection process are listed in *Figure 1*. A total of 23,376 articles were retrieved after the initial search of the chosen electronic databases.

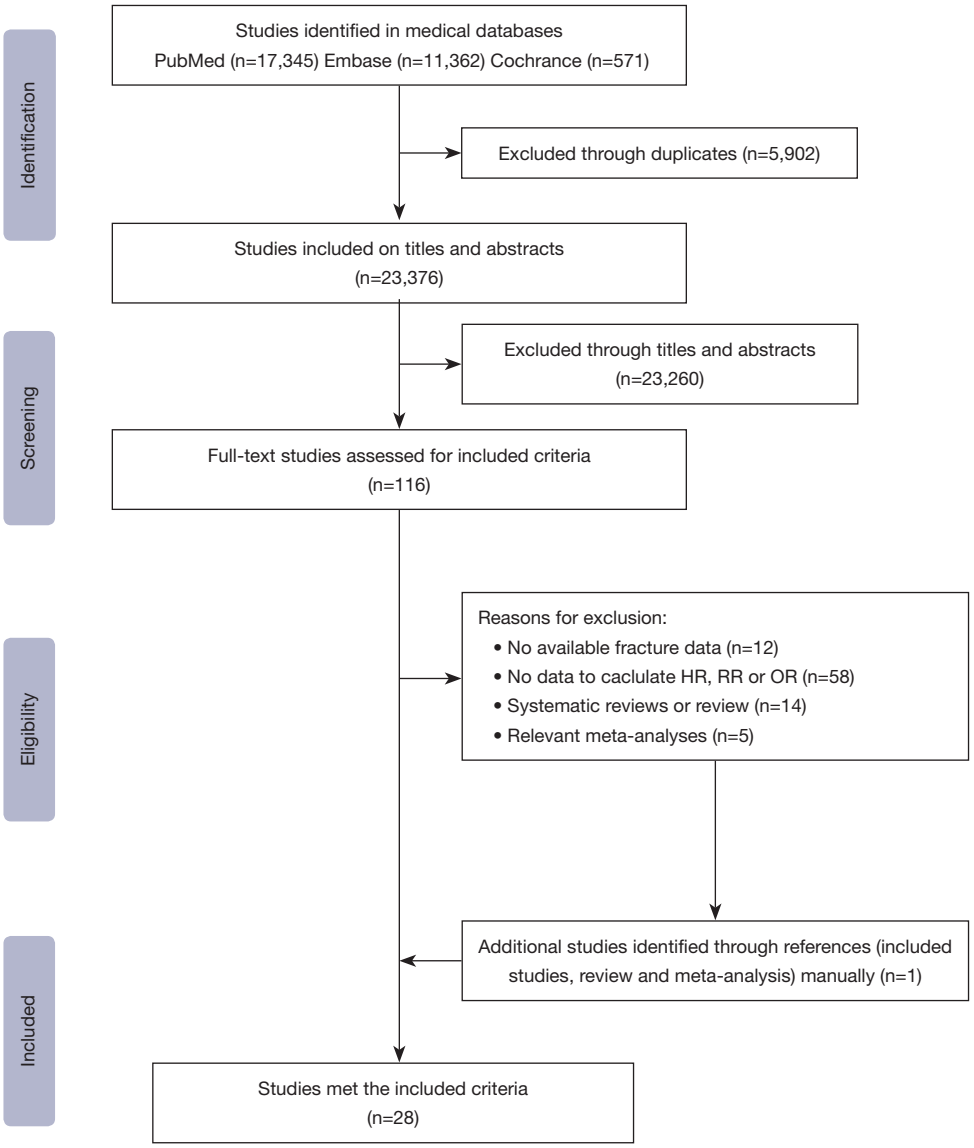

Figure 1 Study selection flow diagram. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Of the 23,376 articles scanned, 23,260 failed the selection criteria, and the 116 remaining articles were subjected to full-text check. Among them, 89 studies were excluded because 11 articles presented no fracture data relating to serum level of HCY; 31 studies were involved in the level of HCY and osteoporosis; 10 studies were involved in the relationship of gene polymorphism and homocysteinemia; 6 were involved in the relationship of gene polymorphism and fracture; 14 were reviews; 5 were meta-analyses; 9 were irrelevant articles; 1 was a study rationale and design; 1 was a survival analysis; and 1 article was retracted (Table S1). Of the included studies, 1 was identified as originating from the reference list of the relevant studies scanned for in the databases. After rigorous selection, eventually, 28 studies involving 60,318 participants (26,508 non-interventions and 33,810 therapeutic interventions) were used for the meta-analysis.

Study characteristic and quality assessment

The detailed characteristics of the studies utilized in the meta-analysis are presented in *Tables 1-3*. Articles were published between 2004 and 2019, with sample sizes ranging of 189–12,064 participants. All included studies

Table 1 Characteristics of the included trials and participants

Study, Year	Country	Journal	Participants	F/M (% women)	Age mean (SD)	HCY	Folate	Vitamin	Follow-up (years)	Fracture type	HR/RR/OR
Lopez *et al.* 2017	Norway	*JBMR*	6,837	1,610/5,227 (76.5%)	62.3 (10.9)	–	+	VB12+6	Mean 10.0 years; median 3.3 [interquartile range (IQR), 2.6–3.5]; 11.1 (IQR, 9.1–12.2) for the extended follow-up	Hip	HR
Torbergsen *et al.* 2015	Norway	*Clinical Nutrition*	189	135/54 (71.4)	82.6 (8.6)	–	+	B6/B12	NR	Hip	OR
Lewerin *et al.* 2014	Sweden	*Osteoporos Int*	760	0/760 (0)	Median 75.3 [70–81]	+	–	–	Mean 5.9 (4.7–7.4)	All; vertebral F	HR
van Wijngaarden *et al.* 2014	Netherlands	*Am J Clin Nutr*	2,919	1,461/1,458 (50.1)	74.1 (6.5)	+	+	B12	> 2	All	HR
Li *et al.* 2014	China	*J Diabetes Invest*	292	191/101 (65.4)	54.3 [41–65]	+	+	B12	NR	All	OR
Urano *et al.* 2014	Japan	*Geriatr Gerontol Int*	663	663/0 (100.0)	NR	-	+	–	Mean 5.1, SD 3.4, max 13	All	HR
Gommans *et al.* 2013	New Zealand	*BMC Geriatrics*	8,164	2,944/5,218 (36.1)	62.6 (12.5)	+	–	–	Mean 3.4 (0.5–10.5)	All; hip; wrist; thoracic spine	RR
Kuroda *et al.* 2013	Japan	*Calcif Tissue Int*	1,475	1,475/0 (100.0)	66.6 (9.0)	+	–	–	≤19	Vertebral	OR
Enneman *et al.* 2012	Netherlands	*Bone*	503	503/0 (100.0)	68.5	+	–	–	7.0 (2.3)	Osteoporotic fracture	HR
Maghraoui *et al.* 2012	Morocco	*J Clin Densitom*	188	188/0 (100.0)	57.9±8.5	+	–	–	NR	Osteoporotic vertebral fracture	RR
Shiraki *et al.* 2011	Japan	*J Bone Miner Metab*	251	251/0 (100.0)	70.5±8.9	+	–	–	3.2±2.0	Osteoporotic vertebral fracture	HR
Armitage *et al.* 2010	UK	*JAMA*	12,064			+	+	Vit B12			RR
Zhu *et al.* 2009	Australia	*Osteoporos Int*	1,213	1,213/0 (100.0)	75.2±2.7	+	–	–	5	Osteoporotic fracture	HR
LeBoff *et al.* 2009	USA	*J Clin Endocrinol Metab*	800	800/0 (100.0)	70.8±6.2	+	–	–	5	Hip	OR
McLean *et al.* 2008	USA	*J Clin Endocrinol Metab*	1,002	603/399 (60.2)	75.3 (4.9)	+	+	VB12+6	14	Hip	HR

Table 1 (*continued*)

Table 1 (*continued*)

Study, Year	Country	Journal	Participants	F/M (% women)	Age mean (SD)	HCY	Folate	Vitamin	Follow-up (years)	Fracture type	HR/RR/OR
Yazdanpanah *et al.* 2007	Netherlands	*Bone*	5,304	3,140/2,164 (59.2)	67.6 (7.75)	+	+	VB12+6	7.4±3.3	Osteoporotic fracture (hip, pelvis and proximal humerus)	HR
Sawka *et al.* 2007	Canada	*Arch Intern Med*	5,522	1,559/3,963 (28.2)	68.8 (7.1)	+	–	–	5	All	HR
Périer *et al.* 2007	France	*Osteoporos Int*	671	671/0 (100.0)	62.2 (9.0)	+	–	–	9.8±1.2	All	HR
Gjesdal *et al.* 2007	Norway	*J Bone Miner Res*	4,766	2,639/2,127 (55.4)	65–67	+	+	Vit B12	13	Hip	HR
Gerdhem *et al.* 2007	Sweden	*J Bone Miner Res*	996	996/0 (100.0)	75 (0)	+	+	Vit B12	7	Osteoporotic fracture	HR
Sato *et al.* 2005	Japan	*Bone*	433	230/203(53.1)	75.4 (5.4)	+	+	Vit B12	9	Osteoporotic fracture (hip)	HR
Ravaglia *et al.* 2005	Italy	*Journal of Gerontology*	702	374/328 (53.3)	73.0 (6.0)	+	+	–	4	Osteoporotic fracture	RR
Dhonukshe-Rutten *et al.* 2005	Netherlands	*J Bone Miner Res*	1,253 (partially overlap with van Meurs' Study)	651/602 (52.0)	76 (6.6)	+	-	Vit B12	3	Osteoporotic fracture	RR
Sato *et al.* 2005*	Japan	*The American Journal of Medicine*	199	199/0 (100.0)	71.0 (5.9)	+	–	–	4.9	Osteoporotic fracture (hip)	HR
van Meurs *et al.* 2004	Netherlands	*N Engl J Med*	2,406	1,292/1,114 (53.7)	73.9 (7.8)	+	–	–	8.1±3.7; 5.7±1.9; 2.7±0.7	Osteoporotic fracture	HR
McLean *et al.* 2004	USA	*N Engl J Med*	1,999	1,174/825 (58.7)	70.0 (7.0)	+	–	–	m 12.3, f 15	Osteoporotic fracture (hip)	HR

*, article retracted. NR, not reported; HR, hazard ratio; RR, risk ratio; OR, odds ratio; HCY, homocysteine; SD, standard deviation.

Table 2 Characteristics of the included studies involving the effectiveness of folate, vitamin B12, and/or vitamin B6 on fractures

Study, year	Country	Treatment/ Placebo	Women No. (%)	Age (y)	Treatment (intake mg/d)			Follow up (years)	Plasma HCY level (μmol/L)		
					Folate	Vit B12	Vit B6		Baseline	Follow-up	Change
Araghi *et al.* 2019	Netherlands	1,298	598 (46.1)	71.0 (68.0–76.0)	0.4	0.5	–	5–7	14.0 (12.8–15.9)	–	-
Garcia Lopez *et al.* 2018	Norway	1,021	370 (36.2)	57.4±9.5	1	–	–	3	9.80±2.90	8.85±2.18	−0.88±2.19
Garcia Lopez *et al.* 2017	Norway	1,708	398 (23.3)	62.7±11.2	0.8	0.4	40	3.2–3.4	12.17±2.78	8.84±3.22	−2.97±3.76
		1,703	390 (22.9)	62.3±10.9	0.8	0.4	–	3.2–3.4	12.06±4.69	9.13±3.71	−2.82±4.57
		1,705	401 (23.5)	62.0±10.9	–	–	40	3.2–3.4	12.30±5.60	12.25±5.04	0.20±4.65
		1,721	421 (24.5)	62.3±10.7	–	–	–	3.2–3.4	12.29±5.06	12.42±5.26	0.46±4.61
van Wijngaarden *et al.* 2014	Netherlands	1,461	736 (50.4)	74.0±6.6	0.4	0.5	–	2–3	14.3 (13.0–16.5)	10.3 (8.9–12.0)	−4.4±3.3
		1,458	725 (49.7)	74.2±6.4	–	–	–	2–3	14.5 (13.0–16.7)	14.3 (12.3–17.0)	−0.2±4.1
Gommans *et al.* 2013	New Zealand	580	–	–	2	0.5	25	0.5–10.5	14.3±8.5	10.5 4.8	–
		584	–	–	–	–	–	0.5–10.5		14.3±6.1	–
Yazdanpanah *et al.* 2007	Netherlands	5,304	–	67.66±7.75	+	+	+	–	–	–	–
Sawka *et al.* 2007	Canada	2,758	796 (28.9)	68.8±7.1	2.5	1	50	–	11.5±?	9.3±?	–
		2,764	763 (27.6)	68.9±6.8	–	–	–	–		12.3±?	–
Armitage *et al.* 2010	UK	6,033	2,052 (17.0)	64.2±8.9	2	1	–	84 mo	13.5±4.8	–	3.3±0.2
		6,031			–	–	–	84 mo			–

+, plus; –, minus; ? standard deviation indicates that standard deviation is unknown. SD, standard deviation; HCY, homocysteine.

Table 3 Characteristics of the included studies evaluating the association of the level of plasma folate, vitamin B12, vitamin B6 with fracture risk

Study, year	Country	Subjects	Women, n (%)	Age mean (SD)	HCY, μmol/L	Folate, nmol/L	Vit B12, pmol/L	Vit B6, nmol/L	Follow-up (years)	Fracture type
Ravaglia *et al.* 2005	Italy	702	374 (53.3)	73.0±6.0	>15	≤9.30	≤190	≤14	4	All
Dhonukshe-Rutten *et al.* 2005	Netherlands	1267	652 (51.5)	76±6.6	>16	–	≤200	–	3	All
Gerdhem *et al.* 2007	Sweden	996	996 (100.0)	75	14.1	–	–	–	7	All
		946	946 (100.0)	75	–	–	308	–	7	All
		978	978 (100.0)	75	–	18	–	–	7	All
Gjesdal *et al.* 2007	Norway	4,482	2,445 (54.6)	65–67	>15	–	–	–	12.6	Hip
		4,490	2,453 (54.6)	65–67	–	<2.9	–	–	12.6	Hip
		4,487	2,450 (54.6)	65–67	–	–	–	–	12.6	Hip
McLean *et al.* 2008	USA	1,002	603 (60.2)	75.3±4.9	>14	<7	<148	<20	4	Hip
Li *et al.* 2014	China	292	190 (65.1)	53.7–55.3 (43.9, 63.5)	Per 5 increase	Per 10 increase	Per 100 increase	–	–	Hip
Torbergsen *et al.* 2015	Norway	189	135 (71.4)	82.6±8.6	–	–	–	–	–	Hip

SD, standard deviation; HCY, homocysteine.

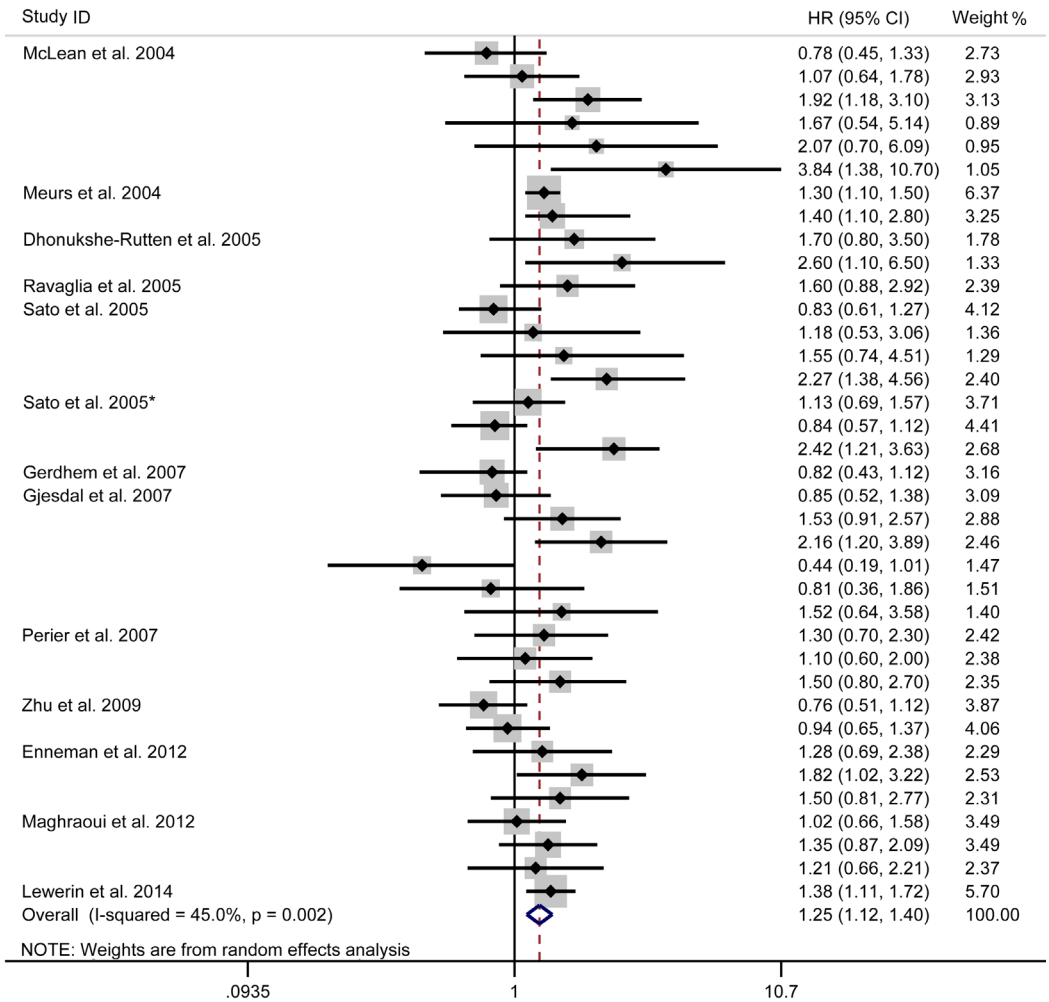

Figure 2 Forest plot of the pooled results involving the association of HCY level with fracture risk. HCY, homocysteine.

were rated as high-quality via the risk of bias assessment (Figure S1).

HCY and fracture risk

A total of 13 trials (5,14,34-44) evaluating the association of HCY level with fracture risk demonstrated that an increased homocysteine level was an independent risk factor for osteoporotic fractures in older persons (HR =1.25, 95% CI: 1.12 to 1.40) (*Figure 2*). Subgroup analysis by excluding two trials without HR data and sensitivity analysis performed by omitting studies 1 by 1 demonstrated consistent results. No significant publication bias was identified using Egger's test (P>0.05, Figure S2). Furthermore, no significant difference was observed in fracture risk between women (5,14,34,37-43) and men (5,14,34,39,44) (HR =1.20, 95% CI: 1.06 to 1.37; 1.41, 95% CI: 1.02 to 1.94) (*Figure 3*).

HCY stratification and fracture risk

For different levels of HCY, the results of meta-analysis generated from 10 studies (5,14,34,36-40,42,43) showed that a HCY level in the highest quartile (or >15 μmol/L) was associated with an increased risk of fracture (HR =1.71, 95% CI: 1.37 to 2.12); however, the second and third quartile generated from 8 studies (5,36,37,39-43) showed no significant association (HR =0.96, 95% CI: 0.80 to 1.16; 1.14, 95% CI: 0.95 to 1.37) (*Figure 4*). Sensitivity analyses performed by excluding 2 trials that provided insufficient data to calculate the HR and its 95% CI or 3 studies involving only participants with stroke, diabetes, and cardiovascular diseases, respectively, demonstrated

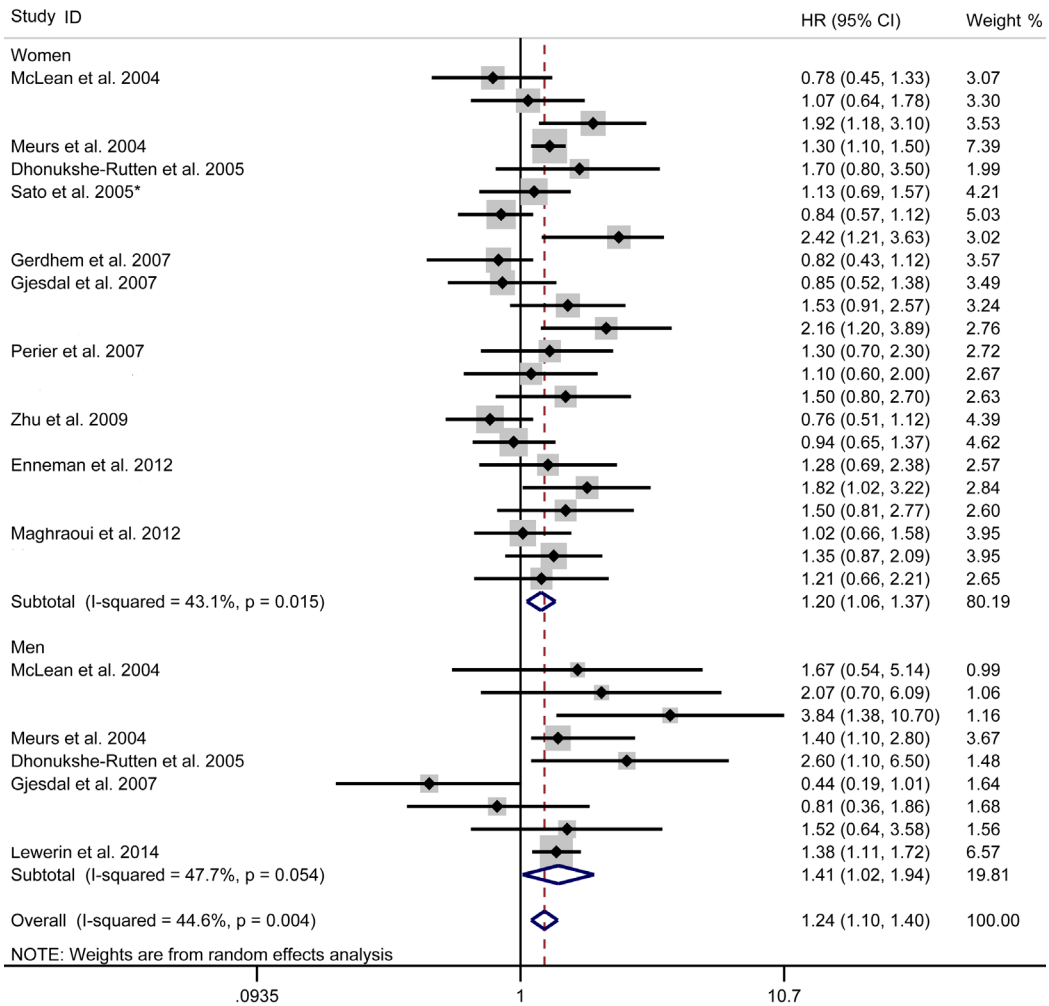

Figure 3 Forest plot of the pooled results involving the association of HCY level with fracture risk of men and women. HCY, homocysteine.

consistent results.

HCY and fracture site

Meta-analysis results generated from 2 studies (38,39) showed that high HCY level was associated with an increased risk of total fractures (HR =1.13, 95% CI: 1.01 to 1.26) as well as nonvertebral and hip fractures (HR =1.36, 95% CI: 1.15 to 1.60; 1.58, 95% CI: 1.24 to 2.00), rather than vertebral fractures (HR =1.13, 95% CI, 0.54 to 2.38) (*Figure 5*). Based on HCY levels, a subgroup analysis for hip fractures demonstrated that the results did not change either with overall data (HR =1.19, 95% CI: 0.96 to 1.48), or in the second, third, and highest quartile (HR =0.77, 95% CI: 0.56 to 1.06; HR =1.18, 95% CI: 0.87 to 1.61; HR =1.53, 95% CI: 1.24 to 1.89, respectively) (Figure S3).

Folate, vitamin B12, vitamin B6 and fracture risk

Our analysis revealed that 4 trials (35,39,45,46), 3 trials (34,35,39), and 2 trials (35,45) evaluating serum folate level, vitamin B12, and vitamin B6 with fracture risk had found that a severe deficiency of folate, instead of vitamin B12 and B6 increased the risk of fracture in older adults (HR =1.46, 95% CI: 1.06 to 2.02; 1.24, 95% CI: 0.79 to 1.95; 1.36, 95% CI: 0.90 to 2.06). Meanwhile, low serum folate, vitamin B12, and vitamin B6 level was not significantly associated with an increased risk of fracture (HR =0.79, 95% CI: 0.56 to 1.12; 1.01, 95% CI: 0.43 to 2.38; 1.16, 95% CI: 0.64 to 2.10) (*Figure 6*).

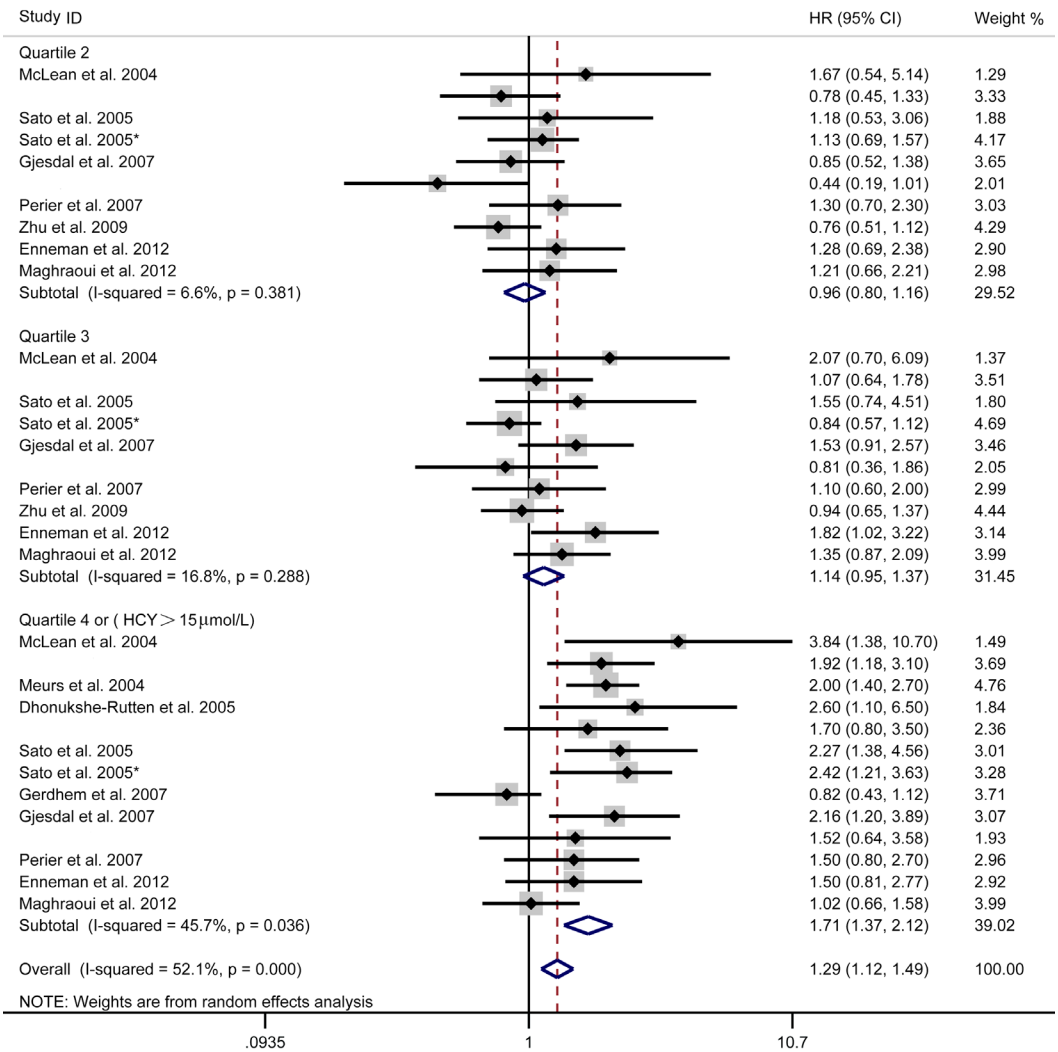

Figure 4 Forest plot of the pooled results involving the association of HCY stratification and fracture risk. HCY, homocysteine.

Folate, vitamin B12, and/or B6 supplementation and fracture risk

In 2 trials (6,21) the results of evaluating participants receiving vitamin B6 supplements *vs.* placebo or no treatment showed no significant association with fracture risk (HR =0.62, 95% CI: 0.28 to 1.39). As shown in *Figure* 7, similar results generated from 2 trials (15,17) involving dual supplementation (folate and vitamin B12) and 3 trials (19-21) with triple supplementation (folate, vitamin B12, and B6) were concluded (HR =1.00, 95% CI: 0.88 to 1.14; HR =0.97, 95% CI: 0.79 to 1.19).

Discussion

In 2004, 2 RCTs (5,14) reported by the *New England Journal of Medicine* indicated that HHCY was associated with higher fracture risk in the older population. However, with the exception of a study conducted in 2005 which was retracted by the *Journal of the American Medical Association* (*JAMA*) in 2016 stating a significant effect on reducing fracture risk in the osteoporotic population by fortification with folate and vitamin B12 (47), a number of prospective randomized cohort intervention trials in the following decade failed to show HCY as an intervention target (15,19-21). The underlying reason for this mixed evidence to date awaits unravelling. Therefore, this meta-analysis was performed to investigate this inconsistence by systematically assessing the association of HCY, folate, vitamin B12, and B6 with fracture risk and whether supplements of B vitamins reduce risk of fracture in the older population.

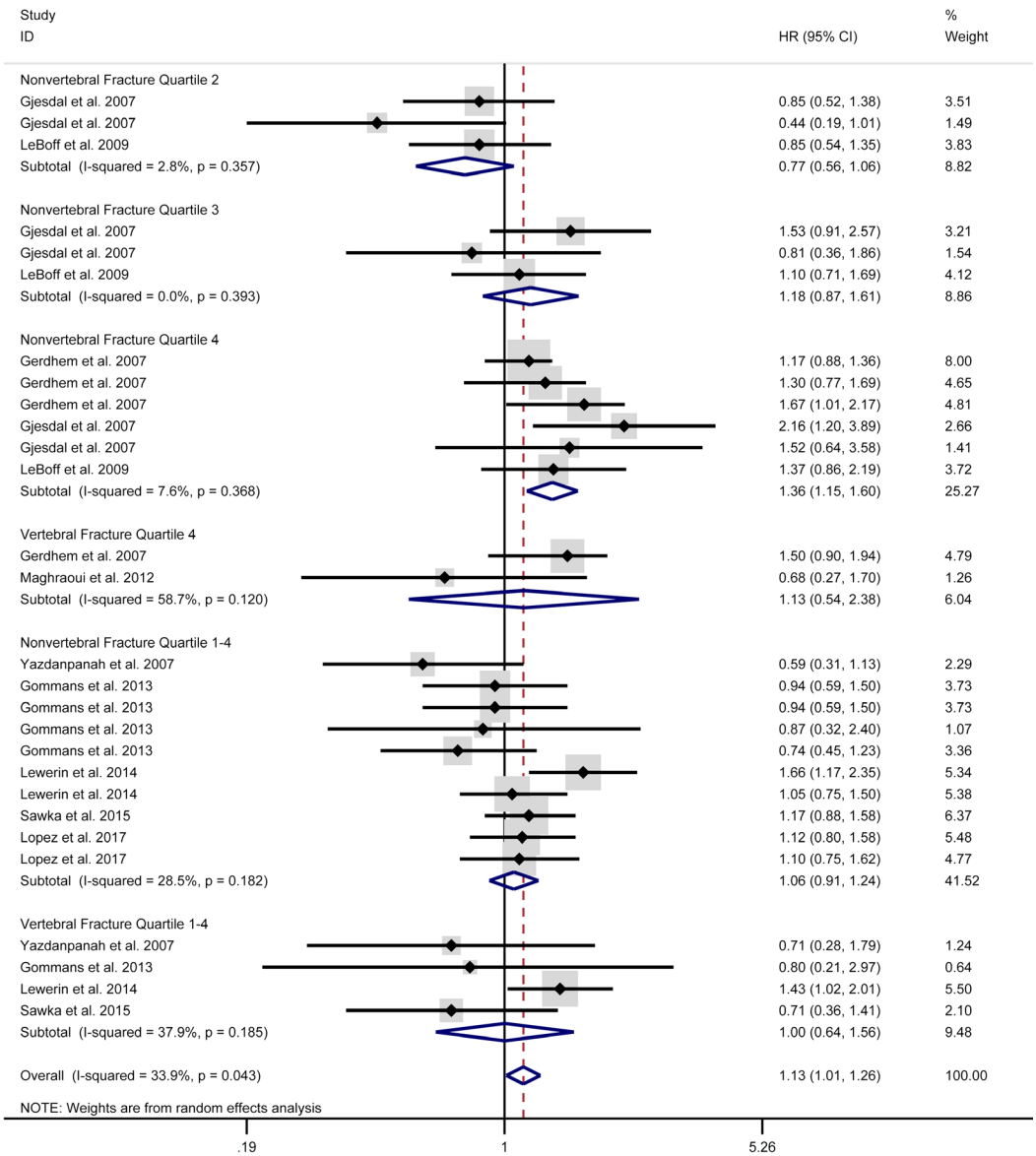

Figure 5 Forest plot of the pooled results involving the association of HCY level and fracture risk at different sites. HCY, homocysteine.

Based on the existing evidence and results in this meta-analysis, intervention of HCY is supposed to have a beneficial effect on osteoporotic fracture in elderly people. The metabolism of HCY has been investigated by multiple studies to uncover possible prevention targets to reduce the serum level of HCY. Among them, folate, vitamin B12, and vitamin B6, the 3 critical enzymes involved in the metabolism of HCY, are considered effective and are subsequently viewed as promising intervention candidates for fracture incidence in older adults thanks to their convenient supplementation by oral intake (18). Evidence has shown that combined folate and vitamin B12 supplementation for 2–4 years could reduce HCY by 2–5 mmol/L (21); while the single application of vitamin B6 has failed to display a significant effect on reducing serum HCY level. Furthermore, the effect on reducing HCY caused by the combined fortification of folate and vitamin B12 is not changed by either the addition or absence of vitamin B6 supplementation (21). In addition, there is limited evidence showing that vitamin B2 may also play a

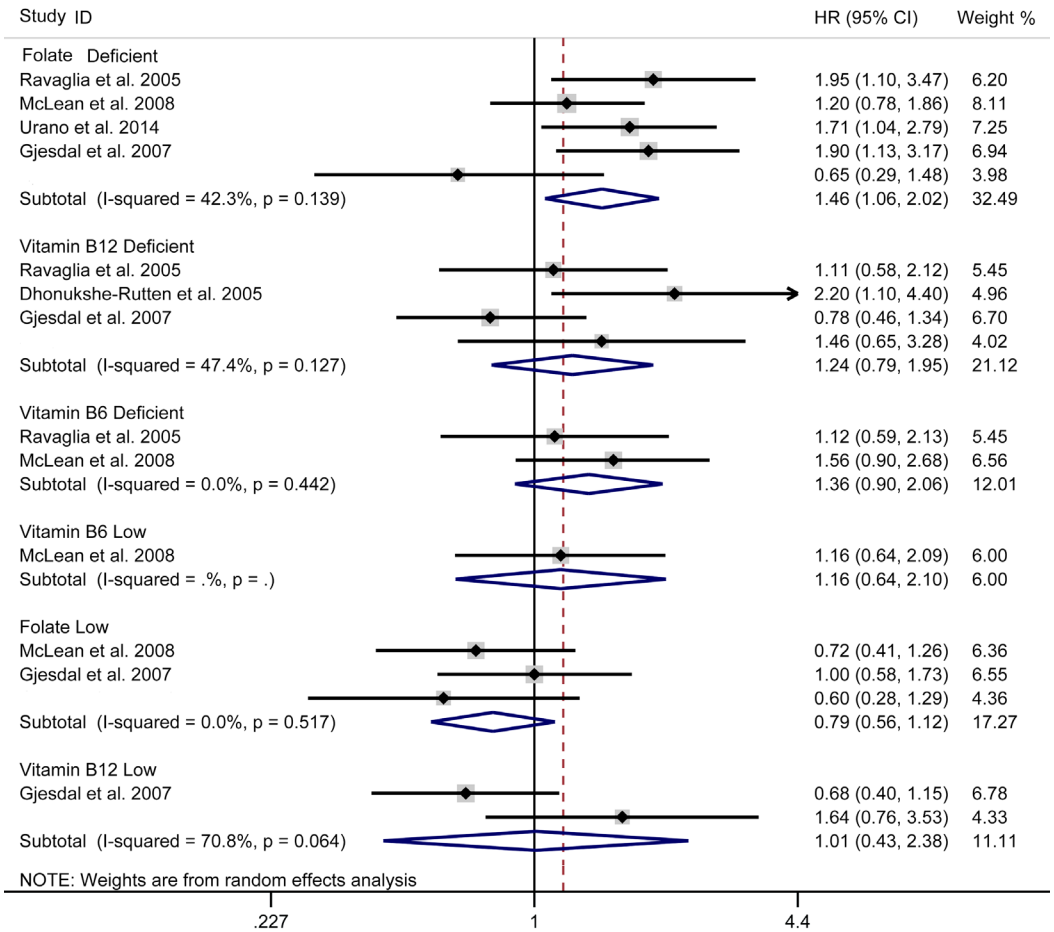

Figure 6 Forest plot of the pooled results involving the association of folate, vitamin B12, vitamin B6, and fracture risk.

role in the metabolism of HCY and can possibly reduce HCY with intervention (27). In this investigation, there was insufficient data concerning vitamin B2 supplementation to support our analysis of its effectiveness.

Encouragingly, a number of studies have confirmed the positive association of folate with BMD, encouraging more large-scale RCTs to validate the beneficial effect of folate fortification on BMD in older adults, although no significant association was observed between BMD and either vitamin B6 or vitamin B12 (6,23,25-27,48). However, researchers have long been perplexed by whether HCY is a culprit or a bystander of fracture in the elderly population. Although the combined application of folate and vitamin B12 can reduce HCY, the present meta-analysis revealed that this intervention was not associated with a lower risk of fracture in older people. A similar phenomenon was noted in a recent large-scale meta-analysis (49) confirming that vitamin D and/or with calcium supplementation was not associated with the decrease of fracture risk in community-dwelling older adults, whereas many trials had previously validated that calcium and vitamin D supplementation could significantly improve BMD (50-52). If folate, vitamin B12, and vitamin B6 share similar mechanisms, the underlying reason that no threshold-effect on fracture risk was observed by intervention of HCY might be that the intervention was not applied to a properly targeted population, subsequently attenuating the effect on reducing HCY level. Based on this hypothesis, we performed a further subgroup analysis to investigate participants with different serum HCY levels. Intention-to-treat participants by serum HCY was separated into 4 groups: the lowest quartile, the second quartile, the third quartile, and the highest quartile. Our subgroup analysis generated encouraging results showing that only the highest quartile of HCY instead of the other 3 quartiles increased the fracture risk in older people. Furthermore, we reviewed all the intervention trials and found that the

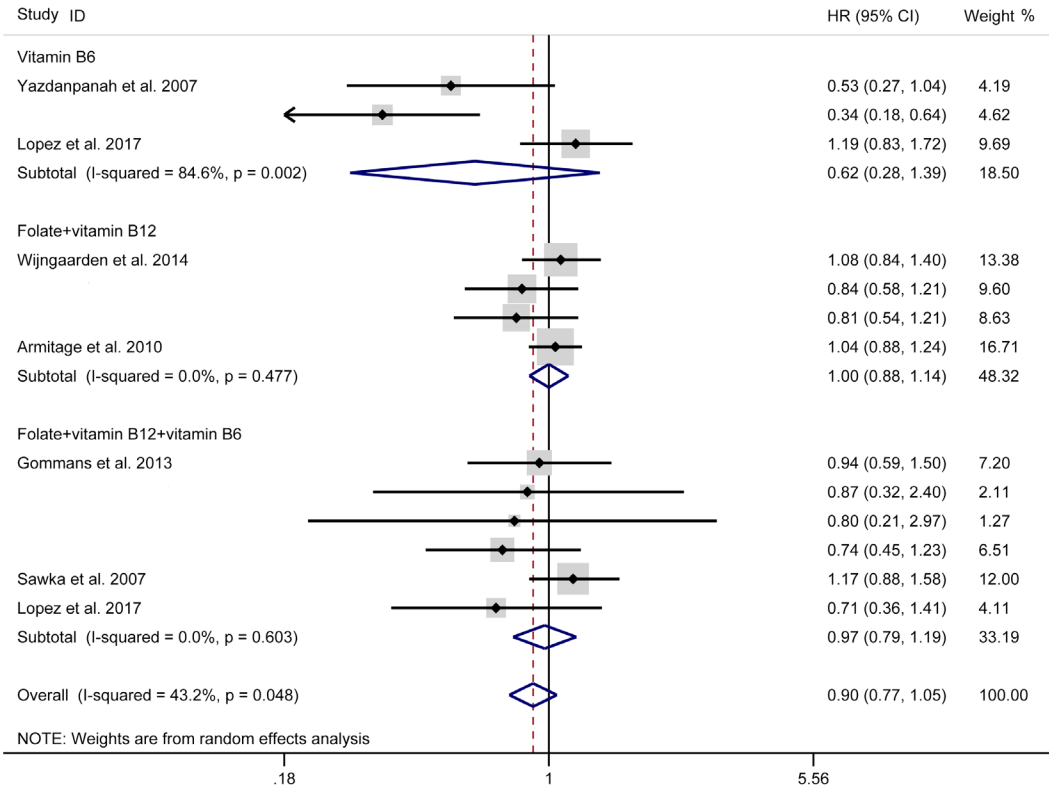

Figure 7 Forest plot of the pooled results involving the association of folate, vitamin B12, and/or B6 supplementation and fracture risk.

mean level of serum HCY, except that of a subgroup in the B-PROOF study, was below 15 μmol/L, which implies that the intervention was not applied to the correct population, leading to no significant effects on decreasing fracture risks (15,19-21,53). Moreover, the subgroup analysis in the B-PROOF study (15,53), with participants over 80 years old and extended follow-up evidence of higher HCY level participants with baseline serum HCY of 15.3 μmol/L (13.6–18.2 μmol/L), supported our hypothesis.

Regretfully, the studies included in our meta-analysis provided no data regarding the extent of lowering HCY. There was 1 study that reported a mean reduction of HCY of 4.5 μmol/L after a 2-year intervention of folate and vitamin B12 (15), while others had a lowering effect of less than 3 μmol/L (6,17,19-21), indicating a potential bias caused by various interventions. Concludingly, all the evidence to date supports the hypothesis that only older people with HCY levels of 15 μmol/L (highest quartile) or above need supplementations of folate and vitamin B12. We also found that the evidence for beneficial effects of vitamin B6 application was not consistent. Garcia Lopez *et al.* stated that intervention with folate and vitamin B12 combined with vitamin B6 did not produce a greater effect on reducing serum HCY. Contrarily, an overdose of vitamin B6 was positively correlated with the fracture incidence possibly owing to its toxic effect (21). Therefore, any supplementation of vitamin B6, based on the current evidence, is not recommended unless a severe deficiency of vitamin B6 has been confirmed.

Based on the dose-dependent effect of HCY reported in a study, 15 μmol/L is before the J point in the standard curve, indicating a significant effect of B vitamins supplementation with a higher threshold of serum HCY level (39). Guidelines regarding the threshold of serum HCY level have been established in cardiovascular disease, cerebrovascular disease, and chronic kidney disease over the past decade (9,10,16). However, consensus can yet be drawn for intervention of HHCY and osteoporotic fractures due to the inconsistent results from studies to date. We therefore strongly encourage more large-scale RCTs focusing on the threshold of HHCY to investigate if a higher threshold would deliver a significant intervention effect on fracture risks in older adults.

The supplementation of both vitamin D and B vitamins has been validated by strong evidence to be ineffective at

lowering fracture risk in community dwelling older people. We tend to believe that in the past decades, researchers have, to some extent, overestimated the beneficial effect of various nutrients including but not only limited to calcium/vitamin D and B vitamins on reducing the risk of osteoporotic fractures in relatively low-risk older people. In the future, we suggest placing increased emphasis on more focalized and economically efficient screening of the high-risk population and the corresponding intervention to achieve a safer and more economical goal, by which the public health resource might be appropriately exploited.

In this study, there were several limitations present. First, no analysis based on evidence to date was able to be carried out to assess the association of people with >15 mmol/L HCY with fracture risk. Hence, no direct evidence is available to validate our hypothesis. Second, HR values from a 199-participants trial by Sato el al. in 2005 was extracted from a graph due to the raw data being inaccessible, which caused some deviation. Besides, some included trials did not test HCY, folate, vitamin B12, and vitamin B6 in all participants, which compromised the analytical power.

Conclusions

This meta-analysis revealed that significantly elevated serum level of HCY is positively associated with fracture incidence in older adults, yet the necessity and threshold for intervention by B vitamins (folate, vitamin B12, and B6) require further large-scale high-quality clinical trials to validate.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://dx.doi.org/10.21037/atm-21-2514.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-2514). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Supplementary

Table S1 The reason and references of excluded full-texts studies

Author	Year	Exclusion reason	Reference
Bailey *et al.*	2015	HCY_Intervention and Osteoporosis	(1-31)
Baines *et al.*	2007		
Bhupathiraju *et al.*	2007		
Blouin *et al.*	2009		
Bozkurt *et al.*	2009		
Bucciarelli *et al.*	2010		
Cagnacci *et al.*	2008		
Dhonukshe-Rutten *et al.*	2003		
Ebesunun *et al.*	2014		
Enneman *et al.*	2015		
Enneman *et al.*	2014		
Garg *et al.*	2014		
Golbahar *et al.*	2004		
Haroon *et al.*	2012		
Karimi *et al.*	2011		
Kim *et al.*	2013		
Kuyumcu *et al.*	2012		
Li *et al.*	2017		
Liu *et al.*	2016		
Morris *et al.*	2005		
Ouzzif *et al.*	2012		
Rehackova *et al.*	2013		
Rejnmark *et al.*	2008		
Rumbak *et al.*	2012		
Salari *et al.*	2014		
Shahab-Ferdows *et al.*	2012		
Tarakida *et al.*	2011		
Vurucu *et al.*	2009		
Weber *et al.*	2016		
Yamada *et al.*	2011		
Yılmaz *et al.*	2009		
Cashman *et al.*	2005	Gene polymorphoism and homosysteinemia	(32-41)
Cook *et al.*	2014		
Guttormsen *et al.*	1996		
Hong *et al.*	2007		
Lacasana *et al.*	2012		
Li *et al.*	2017		
Pandey *et al.*	2013		
Qin *et al.*	2012		
Saito *et al.*	2009		
Tongboonchoo et al	2013		
Bathum *et al.*	2004	Gene polymorphism and Fracture	(42-47)
Chung *et al.*	2012		
Kim *et al.*	2016		
Shiraki *et al.*	2008		
Villadsen *et al.*	2005		
Yazdanpanah *et al.*	2008		
Herrmann *et al.*	2005	No fracture data regarding HCY	(48-58)
Keser *et al.*	2013		
Komulainen-Ebrahim *et al.*	2017		
Kutílek *et al.*	2012		
Lacroix *et al.*	2008		
Meera *et al.*	2010		
Øyen *et al.*	2015		
Rhew *et al.*	2008		
Swart *et al.*	2016		
Tsuchie *et al.*	2016		
Zhu *et al.*	2016		
Ahmadieh H. *et al.*	2011	Review	(59-72)
Bailey *et al.*	2015		
Behera *et al.*	2017		
Clarke *et al.*	2014		
Fratoni *et al.*	2015		
Herrmann *et al.*	2006		
Herrmann *et al.*	2007		
Herrmann *et al.*	2008		
Hiraoka *et al.*	2017		
Mclean *et al.*	2007		
Nieves *et al.*	2012		
Petramala *et al.*	2009		
Saito *et al.*	2006		
Swart *et al.*	2013		
Colson *et al.*	2015	Meta-analysis	(73-77)
Ruan *et al.*	2015		
van Wijngaarden *et al.*	2013		
Yang *et al.*	2012		
Zhang *et al.*	2014		
Ochi *et al.*	2017	Non-research articles	(78-82)
Raisz *et al.*	2004		
Spence *et al.*	2017		
van Meurs *et al.*	2005		
No authors listed	2005		
Bezsmertnyi	2013	No relevance	(83-86)
Lanzoni *et al.*	2017		
Smulders *et al.*	2013		
Tyagi *et al.*	2011		
van Wijngaarden *et al.*	2011	Study rationale and design	(87)
Luo *et al.*	2017	HCY and survival analysis	(88)
Sato *et al.*	2005	Retracted	(89)

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	Study	Items			NOS	Study Design
		Selection	Comparability	Outcome/Exposure		
Observational study	McLean *et al.* 2004	****	**	***	9	Prospective cohort study
	van Meurs *et al.* 2004	****	**	***	9	Prospective cohort study
	Dhonukshe-Rutten *et al.* 2005	****	**	***	9	Prospective cohort study
	Ravaglia *et al.* 2005	****	**	***	9	Prospective cohort study
	Sato *et al.* 2005	****	**	***	9	Cohort study
	Sato *et al.* 2005[$]	****	**	***	9	Prospective control study
	Gerdhem *et al.* 2007	****	**	***	9	Prospective cohort study
	Gjesdal *et al.* 2007	****	**	***	9	Prospective cohort study
	Périer *et al.* 2007	****	**	***	9	Prospective cohort study
	Sawka *et al.* 2007	****	**	***	9	Prospective cohort study
	Yazdanpanah *et al.* 2007	****	**	***	9	Prospective cohort study
	McLean *et al.* 2008	****	**	***	9	Prospective cohort study
	LeBoff *et al.* 2009	****	**	***	9	Case-control study
	Zhu *et al.* 2009	****	**	***	9	Cohort study
	Shiraki *et al.* 2011	****	**	***	9	Prospective cohort study
	El Maghraoui *et al.* 2012	****	**	**	8	Prospective cohort study
	Enneman *et al.* 2012	****	**	***	9	Population-based cohort study
	Kuroda *et al.* 2013	****	**	***	9	Cross-sectional cohort study
	Lewerin *et al.* 2014	****	**	***	9	Prospective cohort study
	Li *et al.* 2014	****	**	**	8	Cross-sectional cohort study
	Urano *et al.* 2014	****	**	***	9	Prospective cohort study
	Torbergsen *et al.* 2015	****	**	**	8	Case-control study

	Study	Items				Jadad	Study Design
		Generation of random sequence	Allocation concealment	Blinding	Withdrawal and dropout		
Experimental study	Armitage *et al.* 2010	**	**	/	*	5	RCT
	Gommans *et al.* 2013	**	**	/	*	5	RCT
	Wijngaarden *et al.* 2014	**	**	/	*	5	RCT
	Lopez *et al.* 2017	**	**	/	*	5	RCT
	NOS, Newcastle-Ottawa scale; RCT, randomized controlled trial						

Figure S1 Quality assessment of included studies. [$], article retracted; *, 1 point; **, 2 points; ***, 3 points; ****, 4 points.

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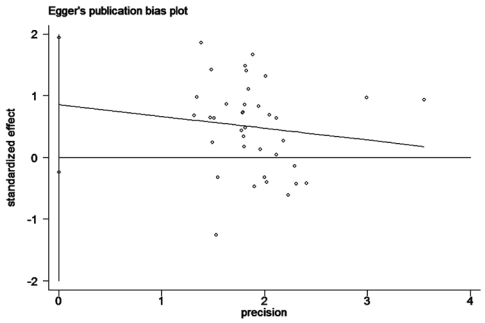

Figure S2 Egger's publicaiton bias plot.

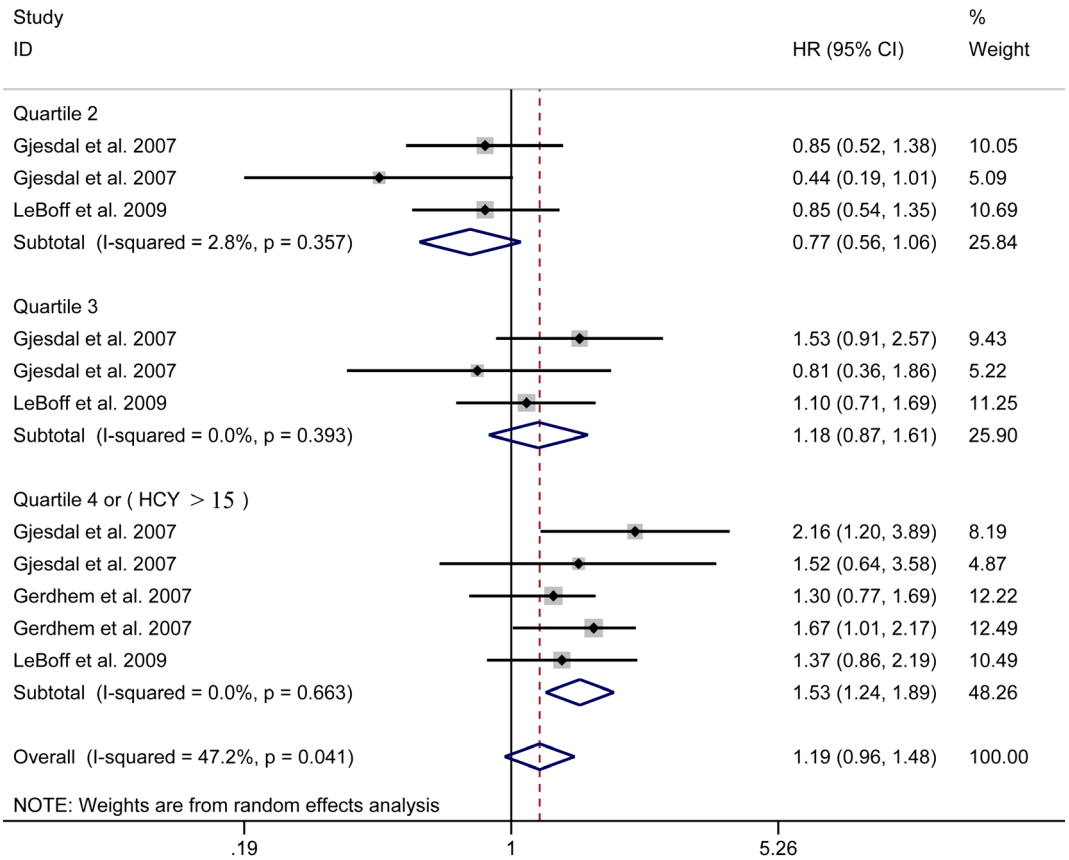

Figure S3 Subgroup analysis for hip fractures demonstrated that the results did not change either with overall data, or in the second, third and highest quartile.

https://dx.doi.org/10.21037/atm-21-2514

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