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Vitamin B_{12} and risk of diabetes: new insight from cross-sectional and longitudinal analyses of the China Stroke Primary Prevention Trial (CSPPT)

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ABSTRACT

Introduction Previous studies in mostly Western populations have yielded conflicting findings on the association of vitamin B_{12} with diabetes risk, in part due to differences in study design and population characteristics. This study sought to examine the vitamin B_{12}–diabetes association in Chinese adults with hypertension by both cross-sectional and longitudinal analyses.

Research design and methods This report included a total of 16 699 participants from the China Stroke Primary Prevention Trial, with pertinent baseline and follow-up data. Diabetes mellitus was defined as either physician-diagnosed diabetes, use of glucose-lowering drugs, or fasting blood glucose (FBG) ≥7.0 mmol/L. New-onset diabetes was defined as any new case of onset diabetes during the follow-up period or FBG ≥7.0 mmol/L at the exit visit.

Results At baseline, there were 1872 (11.2%) patients with diabetes; less than 1.5% had clinical vitamin B_{12} deficiency (<148.0 pmol/L). Over a median follow-up period of 4.5 years, there were 1589 (10.7%) cases of new-onset diabetes. Cross-sectional analyses showed a positive association between baseline vitamin B_{12} levels and FBG levels (β=0.18, 95% CI 0.15 to 0.21) and diabetes (OR=1.16, 95% CI 1.10 to 1.21). However, longitudinal analyses showed no association between baseline vitamin B_{12} and new-onset diabetes or changes in FBG levels. Among a subset of the sample (n=4366) with both baseline and exit vitamin B_{12} measurements, we found a positive association between an increase in vitamin B_{12} and an increase in FBG.

Conclusions In this large Chinese population of patients with hypertension mostly sufficient with vitamin B_{12}, parallel cross-sectional and longitudinal analyses provided new insight into the conflicting findings of previous studies, and these results underscore the need for future studies to consider both baseline vitamin B_{12} and its longitudinal trajectory in order to better elucidate the role of vitamin B_{12} in the development of diabetes. Such findings would have important clinical and public health implications.

Significance of this study

What is already known about this subject?

- Conflicting findings were derived from previous studies on B_{12} and diabetes using either cross-sectional or longitudinal design. Some cross-sectional studies found that B_{12} was positively associated with diabetes, while others reported the opposite results. Several longitudinal studies showed no significant relationship between B_{12} and DM).
- To date, the relationship between B_{12} and DM has remained elusive.

What are the new findings?

- This is the first and largest study of a Chinese population of patients with hypertension (n=16 699) to delineate cross-sectional and longitudinal associations between plasma vitamin B_{12} and diabetes risk.
- The cross-sectional analyses showed a positive association between baseline vitamin B_{12} levels and diabetes; however, longitudinal analyses revealed no association. Among a subset of sample (n=4366) with both baseline and exit B_{12} levels, we found a positive association between an increase in B_{12} and an increase in fasting blood glucose.

How might these results change the focus of research or clinical practice?

- Findings from our cross-sectional and longitudinal analyses underscore the need for future studies to consider both baseline vitamin B_{12} and its longitudinal trajectory in order to better elucidate the role of vitamin B_{12} in the development of diabetes.
- Such findings, if further confirmed, have important clinical and public health implications.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that has reached epidemic levels around the world.[1] In China, there has been a sharp increase in diabetes prevalence in the past few decades, and currently 11.4 million people have diabetes.[2] From both clinical and public health perspectives, there is a critical need to develop

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cost-effective strategies to prevent diabetes. Vitamin B_{12} is a coenzyme in the one-carbon metabolic pathway involved in the synthesis of methionine and pyrimidine and purine bases. Deficiencies in vitamin B_{12} and associated DNA damage and subsequent faulty repair are known to contribute to the development of vascular diseases, cancer, and some birth defects, and can lead to hyperhomocysteinemia. Often related to folic acid deficiency, vitamin B_{12} has been identified as a risk factor for both hypertension and atherosclerosis.[3]

To date, most studies on vitamin B_{12} and DM have been centered on vitamin B_{12} deficiency among existing patients with diabetes. The association between metformin use and low vitamin B_{12} levels has been supported by various levels of evidence.[4] Because ileal vitamin B_{12} absorption is a calcium-dependent process, and metformin is known to have an effect on calcium-dependent membrane action, patients with type 2 diabetes usually developed a marked reduction in serum vitamin B_{12} while being treated with metformin.[5] However, the risks and benefits of vitamin B_{12} on future risk of DM are not clear due to inconsistent results of previous studies. A cross-sectional study in a South Indian population showed that higher vitamin B_{12} levels decreased the risk of DM.[6] Another longitudinal randomized control trial study showed no difference in the incidence of type 2 diabetes mellitus between the vitamin B_{12}-supplemented group as compared with the non-supplemented control group.[7] The current study addresses an important yet controversial topic of whether vitamin B_{12} is associated with DM.

This current study was motivated by the findings of the US National Health and Nutrition Examination Survey (NHANES)[8] which showed that vitamin B_{12} levels in patients with DM without metformin were significantly higher than those in the general population. However, the NHANES is a cross-sectional study, and in order to address whether vitamin B_{12} levels that are higher than the optimal range are a risk factor for developing DM, a prospective cohort study would be required to assess the temporal and dose–response relationship.

In this report, we analyzed a total of 16 699 participants with hypertension from the China Stroke Primary Prevention Trial (CSPPT), with pertinent baseline data and a mean follow-up of 4.5 years. Our primary objective is to perform both cross-sectional and longitudinal analyses with the aim of determining whether the findings of the NHANES could be replicated in a Chinese population, and furthermore whether there is a prospective and dose–response association between baseline vitamin B_{12} levels and risk of new-onset DM. Among a subset of the sample (n=4366) with both baseline and exit vitamin B_{12} measurements, we further analyzed the relationship between the change in vitamin B_{12} levels and the change in fasting blood glucose (FBG) levels from baseline to the exit visit as the secondary objective.

RESEARCH DESIGN AND METHODS

Participants and trial design

All participants provided written, informed consent. A total of 20 702 eligible participants, stratified by the methylenetetrahydrofolate reductase (*MTHFR*) C677T genotypes (CC, CT, or TT), were randomly assigned, in a 1:1 ratio, to one of two treatment groups: a daily oral dose of one tablet containing 10 mg enalapril and 0.8 mg folic acid (the enalapril-folic acid group), or a daily oral dose of one tablet containing 10 mg enalapril only (the enalapril group). Participants were engaged in follow-up visits every 3 months.

A detailed description and the primary results of the CSPPT have been reported elsewhere.[9–11] Briefly, the CSPPT was a multicommunity, randomized, double-blind, controlled trial conducted between May 19, 2008 and August 24, 2013 in 32 communities in China. Eligible participants were men and women aged 45–75 years with hypertension, defined as seated, resting systolic blood pressure (SBP) ≥140 mm Hg or diastolic blood pressure (DBP) ≥90 mm Hg at both the screening and recruitment visit, or who were taking antihypertensive medication. The major exclusion criteria included history of physician-diagnosed stroke, myocardial infarction (MI), heart failure, postcoronary revascularization, or congenital heart disease. According to the CSPPT study protocol people who had long-term B-group vitamin supplementation were excluded, and other B-group vitamins should not be supplemented during the study period.

This report included 16 699 men and women with hypertension from the CSPPT with baseline vitamin B_{12} data and pertinent baseline and follow-up data on diabetes status and covariables. As illustrated in the flow chart (online supplementary figure S1), the final analyses excluded participants with missing values for baseline vitamin B_{12}, baseline FBG, exit FBG and with any missing data on the follow-up questionnaire. We also randomly selected a subset of the population (n=4366) to detect changes in vitamin B_{12} at the exit visit (online supplementary table S1).

Outcomes

Patients were classified as diabetic if they self-reported a physician diagnosis, or were using glucose-lowering medication, or when their FBG ≥7.0 mmol/L at baseline.[12] New-onset diabetes was defined as a self-reported physician diagnosis, or use of glucose-lowering drugs during the follow-up period, or when FBG changed from <7.0 mmol/L at baseline to ≥7.0 mmol/L at the last study (exit) visit.

Covariables

Covariables included known or suspected factors associated with vitamin B_{12} and/or DM based on existing literature, including our own studies in the CSPPT, specifically age, sex, *MTHFR* gene C677T polymorphisms, SBP and DBP at baseline, mean SBP and DBP during the treatment

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period, body mass index (BMI), study center, serum concentrations of folate, total homocysteine (tHcy), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), smoking status, alcohol consumption status, self-reported meat consumption and medication use. Information on meat consumption was self-reported at baseline using a simple abbreviated semi-quantitative Food Frequency Questionnaire. Participants were asked to report how often, on average, they eat meat every week. Possible response categories included 'never', '1–2 times/week', '3–5 times/week' and 'every day'. Vitamin B_{12} deficiency was defined as vitamin B_{12} <148.0 pmol/L.[13]

Laboratory assays

For biochemical analyses, 20 mL blood samples were collected between 7:00 and 9:00 after an overnight fast (at least 8 hours). Serum samples were separated, aliquoted and subsequently stored at –80°C until analysis. Plasma vitamin B_{12} at baseline and folate at baseline and the exit visit were measured using a chemiluminescent immunoassay at the commercial lab (New Industrial, Shenzhen, China). tHcy, fasting lipids and FBG at baseline and the exit visit were measured using automatic clinical analyzers (Beckman Coulter, California, USA) at the core lab of the National Clinical Research Center for Kidney Disease (Nanfang Hospital, Guangzhou, China).

Statistical analysis

Descriptive data are presented as mean (SD) or median values with IQR in parentheses or proportions, as appropriate, for population characteristics according to baseline vitamin B_{12} quartiles. The significance of differences in population characteristics between groups was computed using two-sample t-tests, signed-rank tests, or χ^2 tests for continuous and categorical variables.

Logistic regression models were used to estimate the ORs and their 95% CIs of diabetes, given the exact onset of diabetes was not known and many new-onset DM cases were detected by fasting glucose levels at the exit visit. All analyses were conducted with adjustments for covariables. Finally, subgroup analyses were performed to evaluate possible effect modifications by the covariables on the association between vitamin B_{12} and DM, including sex (male vs female), age (<60 vs ≥60 years), *MTHFR* C677T polymorphism (CC vs CT vs TT), SBP (<160.0 vs ≥160.0 mm Hg), DBP (<90 vs ≥90 mm Hg), mean SBP during the treatment period (<140.0 vs ≥140.0 mm Hg), mean DBP during the treatment period (<90 vs ≥90 mm Hg), BMI (<25 vs ≥25 kg/m^2), study center (Anqing vs Lianyungang), folate (<8 vs ≥8 ng/mL), tHcy (<12.5 vs ≥12.5 µmol/L), TC (<5.5 vs ≥5.5 mmol/L), TG (<1.5 vs ≥1.5 mmol/L), HDL-C (<1.3 vs ≥1.3 mmol/L) and treatment group (enalapril vs enalapril-folic acid). A two-tailed p<0.05 was considered significant in all analyses. All statistical analyses were performed using R software, V.3.6.0 (http://www.R-project.org/, accessed April 26, 2019).

RESULTS

Study participants and baseline characteristics

Study participants had an average age of 60.0 years (SD 7.4), 6713 were male (40.2%) and 9986 were female (59.8%) (online supplementary table S2). Participants had an average vitamin B_{12} level of 295.9 pmol/L (SD 91.5), 257 (1.5%) participants had vitamin B_{12} deficiency, while 10 468 (63.3%) participants did not consume meat. The mean FBG level was 5.8 mmol/L (SD 1.7) at baseline, and the exit mean FBG level was 6.3 mmol/L (SD 2.0). At baseline, 1872 (11.2%) participants had DM, and at the exit visit there were 1589 (10.7%) cases of new-onset DM. When stratified by baseline vitamin B_{12} quartiles, FBG levels were the highest (6.0 mmol/L (SD 2.1)) in the fourth quartile (Q4). Table 1 shows that the average age and BMI of the participants in Q4 were lower than those of the other groups, but TC and TG levels were higher than the other quartiles.

Cross-sectional analysis on baseline vitamin B_{12} and DM

From the cross-sectional analysis, vitamin B_{12} was found to be positively associated with DM (OR=1.35, 95% CI 1.26 to 1.44, p<0.001) at baseline (table 2). After stratifying by vitamin B_{12} quartiles, participants in Q4 were found to have the highest risk (OR=1.68, 95% CI 1.43 to 1.98, p<0.001). Also, there was a positive association between vitamin B_{12} and FBG (β=0.14, 95% CI 0.11 to 0.17, p<0.001) (table 3). After stratifying by relevant covariables, we discovered interactions between sex, TC levels and vitamin B_{12} with baseline DM and FBG (online supplementary figure S2, online supplementary figure S3). No interaction was found between vitamin B_{12} and plasma folic acid or tHcy.

Longitudinal analyses on baseline vitamin B_{12} and new-onset DM

Longitudinal analyses did not show an association between baseline vitamin B_{12} and new-onset DM (OR=0.97, 95% CI 0.90 to 1.04, p=0.346) (table 2), change in FBG (β=–0.01, 95% CI –0.04 to 0.02, p=0.602), or exit FBG (β=–0.01, 95% CI –0.04 to 0.02, p=0.602) after making additional adjustments for baseline FBG (table 3). After stratifying by relevant covariables, no interaction was found between vitamin B_{12} with new-onset DM, exit FBG or change in FBG (online supplementary figure S2, online supplementary figure S3).

Longitudinal analyses on change in vitamin B_{12} levels and change in FBG levels

Among a subset of the sample (n=4366) with both baseline and exit vitamin B_{12} measurements, we further analyzed the relationship between change in vitamin B_{12} levels and change in FBG levels from the baseline to the exit visit. We found a dose–response relationship between

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Table 1 Baseline and follow-up characteristics of the study participants by baseline B_{12} quartiles

Variables	Total	Baseline vitamin B_{12} quartiles (pmol/L)				P value
		Q1 (<232.3)	Q2 (232.3–279.4)	Q3 (279.4–349.8)	Q4 (≥349.8)	
n	16699	4175	4173	4176	4175	
Age (years)	60.0 (7.4)	60.4 (7.4)	60.1 (7.5)	59.7 (7.4)	59.9 (7.3)	<0.001
Male	6713 (40.2%)	1833 (43.9%)	1632 (39.1%)	1661 (39.8%)	1587 (38.0%)	<0.001
BMI (kg/m^2)	25.0 (3.7)	24.9 (3.6)	25.2 (3.7)	25.2 (3.7)	24.8 (3.6)	<0.001
Total cholesterol (mmol/L)	5.5 (1.2)	5.3 (1.1)	5.5 (1.2)	5.6 (1.2)	5.7 (1.3)	<0.001
Triglycerides (mmol/L)	1.7 (1.2)	1.6 (0.9)	1.6 (0.9)	1.7 (1.0)	1.7 (1.8)	<0.001
HDL-C (mmol/L)	1.3 (0.4)	1.3 (0.3)	1.3 (0.4)	1.3 (0.4)	1.4 (0.4)	<0.001
ALT (U/L)	49.1 (5.8)	48.3 (5.6)	49.1 (5.8)	49.6 (5.8)	49.5 (5.8)	<0.001
AST (U/L)	26.1 (36.5)	24.8 (9)	24.6 (9.1)	25.9 (11.1)	29.2 (70.8)	<0.001
FBG (mmol/L)	5.8 (1.7)	5.6 (1.3)	5.8 (1.5)	5.9 (1.7)	6.0 (2.1)	<0.001
Exit FBG (mmol/L)	6.3 (2.0)	6.1 (1.7)	6.2 (2.0)	6.3 (2.1)	6.4 (2.2)	<0.001
Folate (ng/mL)	8.0 (5.6–10.4)	7.5 (5.2–9.9)	7.7 (5.4–10)	8.1 (5.7–10.4)	8.7 (6.1–11.1)	<0.001
B_{12} (pmol/L)	277.8 (231.7–345.7)	205.7 (184.7–219.8)	254.8 (243.8–266.6)	308.7 (292.6–327.5)	409.5 (375.6–460.2)	<0.001
Homocysteine (µmol/L)	12.5 (10.4–15.4)	13.8 (11.3–17.5)	12.7 (10.6–15.9)	12.1 (10.3–14.6)	11.6 (9.8–13.9)	<0.001
eGFR (mL/min per 1.73 m^2)	93.6 (13.0)	93.8 (12.5)	93.6 (13.0)	94.0 (12.7)	92.8 (13.6)	<0.001
SBP (mm Hg)	167.2 (20.4)	165.7 (20.1)	167.4 (20.4)	168.4 (20.8)	167.4 (20.3)	<0.001
DBP (mm Hg)	94.2 (11.9)	93.2 (12.0)	94.2 (11.8)	94.8 (11.8)	94.4 (11.9)	<0.001
Mean SBP during the treatment period (mm Hg)	139.0 (10.6)	139.1 (10.8)	139.0 (10.5)	138.9 (10.4)	138.8 (10.5)	0.641
Mean DBP during the treatment period (mm Hg)	82.8 (7.2)	82.6 (7.4)	82.8 (7.3)	83.0 (7.1)	82.7 (7.1)	0.058
MTHFR C677T						<0.001
CC	4529 (27.1%)	1047 (25.1%)	1060 (25.4%)	1163 (27.8%)	1259 (30.2%)	
CT	8185 (49.0%)	1962 (47.0%)	2015 (48.3%)	2121 (50.8%)	2087 (50.0%)	
TT	3985 (23.9%)	1166 (27.9%)	1098 (26.3%)	892 (21.4%)	829 (19.9%)	
Treatment group						0.814
Enalapril	8369 (50.1%)	2076 (49.7%)	2098 (50.3%)	2080 (49.8%)	2115 (50.7%)	
Enalapril-folic acid	8330 (49.9%)	2099 (50.3%)	2075 (49.7%)	2096 (50.2%)	2060 (49.3%)	
Center						<0.001
Anqing	3905 (23.4%)	869 (20.8%)	712 (17.1%)	946 (22.7%)	1378 (33.0%)	
Lianyungang	12794 (76.6%)	3306 (79.2%)	3461 (82.9%)	3230 (77.3%)	2797 (67.0%)	
Smoking status						<0.001
Never	11613 (69.6%)	2778 (66.6%)	2941 (70.5%)	2917 (69.9%)	2977 (71.3%)	

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Table 1 Continued

Variables	Total	Baseline vitamin B_{12} quartiles (pmol/L)				P value
		Q1 (<232.3)	Q2 (232.3–279.4)	Q3 (279.4–349.8)	Q4 (≥349.8)	
Former	1259 (7.5%)	322 (7.7%)	271 (6.5%)	313 (7.5%)	353 (8.5%)	
Current	3820 (22.9%)	1072 (25.7%)	960 (23.0%)	945 (22.6%)	843 (20.2%)	
Alcohol drinking						0.334
Never	11596 (69.5%)	2858 (68.5%)	2959 (70.9%)	2878 (68.9%)	2901 (69.5%)	
Former	1145 (6.9%)	290 (7.0%)	279 (6.7%)	292 (7.0%)	284 (6.8%)	
Current	3947 (23.7%)	1022 (24.5%)	933 (22.4%)	1005 (24.1%)	987 (23.7%)	
Meat consumption						<0.001
Never	10468 (63.3%)	2788 (68.0%)	2671 (64.6%)	2562 (61.7%)	2447 (59.1%)	
1–2 times per week	4642 (28.1%)	1069 (26.1%)	1131 (27.4%)	1186 (28.6%)	1256 (30.3%)	
3–5 times per week	978 (5.9%)	173 (4.2%)	227 (5.5%)	292 (7.0%)	286 (6.9%)	
Every day	442 (2.7%)	71 (1.7%)	106 (2.6%)	112 (2.7%)	153 (3.7%)	
Medication use						
Antidiabetic drugs	260 (1.6%)	33 (0.8%)	46 (1.1%)	73 (1.7%)	108 (2.6)%	<0.001
Lipid-lowering drugs	141 (0.8%)	32 (0.8%)	36 (0.9%)	32 (0.8%)	41 (1%)	0.667
Antihypertensive drugs	7813 (46.8%)	1921 (46%)	1956 (46.9%)	1943 (46.5%)	1993 (47.7%)	0.45

Data are n (%), mean (SD), and median (IQR).
The significance of differences in population characteristics between groups was computed using signed-rank tests, or χ^2 tests, for continuous and categorical variables.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; *MTHFR*, methylenetetrahydrofolate reductase; SBP, systolic blood pressure.

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Table 2 Cross-sectional and longitudinal association between baseline vitamin B_{12} levels and diabetes (DM) and new-onset DM

Baseline vitamin B_{12}	Cross-sectional*				Longitudinal†			
	DM				New-onset DM			
	n	Cases (%)	Non-adjusted	Adjusted	n	Cases (%)	Non-adjusted	Adjusted
			OR (95% CI), p value	OR (95% CI), p value			OR (95% CI), p value	OR (95% CI), p value
Continuous (per IQR)	16 699	1872 (11.2)	1.36 (1.28 to 1.44), <0.001	1.35 (1.26 to 1.44), <0.001	14 827	1589 (10.7)	1.02 (0.95 to 1.09), 0.572	0.97 (0.90 to 1.04), 0.346
Quartiles								
Q1	4175	353 (8.5)	1	1	3707	391 (10.5)	1	1
Q2	4173	404 (9.7)	1.15 (0.99 to 1.34), 0.071	1.02 (0.87 to 1.21), 0.788	3706	383 (10.3)	0.99 (0.85 to 1.14), 0.849	0.94 (0.80 to 1.10), 0.447
Q3	4176	493 (11.8)	1.47 (1.27 to 1.69), <0.001	1.23 (1.04 to 1.44), 0.013	3707	417 (11.2)	1.07 (0.92 to 1.24), 0.387	0.98 (0.83 to 1.14), 0.768
Q4	4175	622 (14.9)	1.83 (1.59 to 2.11), <0.001	1.68 (1.43 to 1.98), <0.001	3707	398 (10.7)	1.02 (0.88 to 1.18), 0.820	0.91 (0.77 to 1.07), 0.244
P for trend			<0.001	<0.001			0.582	0.341

*Adjusted for age, sex, *MTHFR* gene C677T polymorphisms, SBP and DBP at baseline, body mass index, study center, serum concentrations of folate, homocysteine, total cholesterol, triglycerides, and high-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, smoking status, alcohol consumption status, meat consumption, lipid-lowering drugs and antihypertensive drugs.
†Adjusted for age, sex, *MTHFR* gene C677T polymorphisms, SBP and DBP at baseline, mean SBP and DBP during the treatment period, body mass index, study center, baseline serum concentrations of folate, homocysteine, fasting blood glucose, total cholesterol, triglycerides, and high-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, treatment group, smoking status, alcohol consumption status, meat consumption, lipid-lowering drugs and antihypertensive drugs.
DBP, diastolic blood pressure; DM, diabetes mellitus; *MTHFR*, methylenetetrahydrofolate reductase; SBP, systolic blood pressure.

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Table 3 Cross-sectional and longitudinal association between baseline vitamin B_{12} and baseline FBG, exit FBG and change in FBG (ΔFBG)

	Cross-sectional*			Longitudinal†					
		Baseline FBG			Exit FBG			ΔFBG	
		Non-adjusted	Adjusted		Non-adjusted	Adjusted		Non-adjusted	Adjusted
Baseline vitamin B_{12}	Mean (SD)	β (95% CI), p value	β (95% CI), p value	Mean (SD)	β (95% CI), p value	β (95% CI), p value	Mean (SD)	β (95% CI), p value	β (95% CI), p value
Continuous (per IQR)	5.8 (1.7)	0.16 (0.13 to 0.19), <0.001	0.14 (0.11 to 0.17), <0.001	6.3 (2.0)	0.13 (0.09 to 0.17), <0.001	−0.01 (−0.04 to 0.02), 0.602	0.5 (1.6)	−0.02 (−0.05 to 0.01), 0.163	−0.01 (−0.04 to 0.02), 0.602
Quartiles									
Q1	5.6 (1.3)	0	0	6.1 (1.7)	0	0	0.5 (1.5)	0	0
Q2	5.8 (1.5)	0.11 (0.04 to 0.18), 0.002	0.03 (−0.04 to 0.09), 0.381	6.2 (2.0)	0.07 (−0.01 to 0.16), 0.094	−0.01 (−0.08 to 0.05), 0.736	0.5 (1.7)	−0.04 (−0.11 to 0.03), 0.241	−0.01 (−0.08 to 0.05), 0.696
Q3	5.9 (1.7)	0.23 (0.16 to 0.31), <0.001	0.13 (0.06 to 0.19), <0.001	6.3 (2.1)	0.19 (0.10 to 0.28), <0.001	0.00 (−0.06 to 0.07), 0.935	0.5 (1.7)	−0.05 (−0.12 to 0.02), 0.144	−0.00 (−0.07 to 0.06), 0.979
Q4	5.9 (2.1)	0.31 (0.24 to 0.38), <0.001	0.26 (0.20 to 0.33), <0.001	6.4 (2.2)	0.23 (0.14 to 0.32), <0.001	−0.03 (−0.10 to 0.03), 0.325	0.4 (1.7)	−0.05 (−0.12 to 0.02), 0.168	−0.03 (−0.10 to 0.03), 0.316
P for trend		<0.001	<0.001		<0.001	0.462		0.162	0.405

*Adjusted for age, sex, *MTHFR* gene C677T polymorphisms, SBP and DBP at baseline, body mass index, study center, serum concentrations of folate, homocysteine, total cholesterol, triglycerides, and high-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, smoking status, alcohol consumption status, meat consumption, lipid-lowering drugs and antihypertensive drugs.
†Adjusted for age, sex, *MTHFR* gene C677T polymorphisms, SBP and DBP at baseline, mean SBP and DBP during the treatment period, body mass index, study center, baseline serum concentrations of folate, homocysteine, FBG, total cholesterol, triglycerides, and high-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, treatment group, smoking status, alcohol consumption status, meat consumption, lipid-lowering drugs and antihypertensive drugs.
DBP diastolic blood pressure; FBG, fasting blood glucose; *MTHFR*, methylenetetrahydrofolate reductase; SBP, systolic blood pressure.

change in vitamin B_{12} and change in FBG levels (table 4, figure 1).

DISCUSSION

This is the first time that the relationship between vitamin B_{12} and DM has been explored in a Chinese population of patients with hypertension via both cross-sectional and longitudinal analyses. We confirmed the findings of the NHANES, showing a cross-sectional positive association between vitamin B_{12} and DM at baseline in this Chinese population. Furthermore, our longitudinal analyses demonstrated that there was no association between baseline vitamin B_{12} levels and new-onset DM risk. Our study has contributed new insights on the vitamin B_{12} and DM association and has helped to explain inconsistent findings in previous studies.

Vitamin B_{12} and DM association depends on population characteristics

Most previous studies on the association of vitamin B_{12} and DM were centered on vitamin B_{12} deficiency among existing patients with DM with the use of metformin. The association between metformin use and low vitamin B_{12} levels has been supported by various levels of evidence.[4] Most of those studies were conducted in older populations, where vitamin B_{12} deficiency is more likely.[8] In contrast, our study was conducted in a Chinese population of patients with hypertension who were relatively young (45–75 years at baseline), mostly free from DM at baseline, and mostly vitamin B_{12} sufficient. Only 6.9% of the study participants with DM reported using metformin (online supplementary figure S4).

Vitamin B_{12} and DM association depends on the study design and type of analyses

In a Mendelian randomization study, Moen *et al*[14] found that vitamin B_{12} may have a causal effect on fasting glucose and one potential mechanism could be an effect of vitamin B_{12} on cell cycle and proliferation of pancreatic β cells, resulting in improved insulin secretion among individuals with higher vitamin B_{12} concentrations. However, in other cross-sectional analyses, Jayashri *et al*[6] found that the levels of vitamin B_{12} decreased with increasing severity of glucose tolerance. Margalit *et al*[15] found no significant difference in blood sugar between the vitamin B_{12}-deficient group and the non-deficient group. In longitudinal analyses and randomized trials, Looker *et al*[16] found that vitamin B_{12} was positively associated with all-cause mortality and death from diabetes/nephropathy. Song *et al*[7] found that daily supplementation with folic acid and vitamins B_6 and B_{12} did not reduce the risk of developing type 2 diabetes among women at high risk for cardiovascular diseases (CVD). Kwok *et al*[17] found that vitamin B_{12} supplementation did not prevent cognitive decline in older patients with diabetes with borderline vitamin B_{12} status. In a systematic review, Rafnsson *et al*[18] found that current data do not support vitamin B_{12}

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Table 4 Longitudinal analyses on change in vitamin B_{12} (ΔB_{12}) and change in' FBG (ΔFBG) from baseline to exit visit*

Exposure			ΔFBG (µmol/L)			
			Non-adjusted model		Adjusted model†	
ΔVitamin B_{12}	n	Mean (SD)	β (95% CI)	P value	β (95% CI)	P value
Continuous (per IQR)	4366	−15.4 (299.5)	0.02 (0.00 to 0.04)	0.018	0.01 (0.00 to 0.02)	0.144
Quartiles						
Q1	1092	−149.1 (155.3)	0		0	
Q2	1091	−38.4 (13.5)	0.05 (−0.09 to 0.19)	0.479	−0.02 (−0.14 to 0.11)	0.789
Q3	1091	6.5 (13.5)	0.23 (0.09 to 0.37)	0.001	0.11 (−0.01 to 0.24)	0.082
Q4	1092	119.2 (545.2)	0.34 (0.20 to 0.48)	<0.001	0.20 (0.08 to 0.33)	0.002
P for trend				<0.001		<0.001

*This subsample included a total of 4366 subjects with both baseline and exit FBG and vitamin B_{12} measurements.
†Adjusted for age, sex, *MTHFR* gene C677T polymorphisms, SBP and DBP at baseline, mean SBP and DBP during the treatment period, body mass index, study center, baseline serum concentrations of folate, homocysteine, FBG, B_{12}, total cholesterol, triglycerides, and high-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, treatment group, smoking status, alcohol consumption status, meat consumption, lipid-lowering drugs and antihypertensive drugs.
DBP, diastolic blood pressure; FBG, fasting blood glucose; *MTHFR*, methylenetetrahydrofolate reductase; SBP, systolic blood pressure.

supplementation in reducing the risk of cardiovascular diseases or diabetes.

Our study was the first to perform and report findings from both cross-sectional and longitudinal analyses in the same population. In the cross-sectional analysis, we found an independent, positive association between baseline vitamin B_{12} levels and DM and FBG. These results persisted even after we adjusted for relevant covariables. This finding is consistent with the NHANES study.[8] In the longitudinal analyses, we did not find any association between baseline vitamin B_{12} and new-onset DM risk. This finding is consistent with the Women's Antioxidant and Folic Acid Cardiovascular Study, where women aged ≥40 years with a history of cardiovascular disease, who were free of DM at baseline, were supplemented with either a combination pill consisting of folic acid, pyridoxine and vitamin B_{12}, or a placebo. After a median follow-up of 7.3 years, no difference in incident type 2 diabetes mellitus was found between the two groups.[7] Another longitudinal study[19] in Japan also reported similar null results. Taken together, our longitudinal analyses and that of others did not support an association between vitamin B_{12} and new-onset DM. These findings underscore that cross-sectional associations need to be confirmed by prospective studies and clinical trials, because the former is more likely to be subject to many drawbacks, including reverse causality.

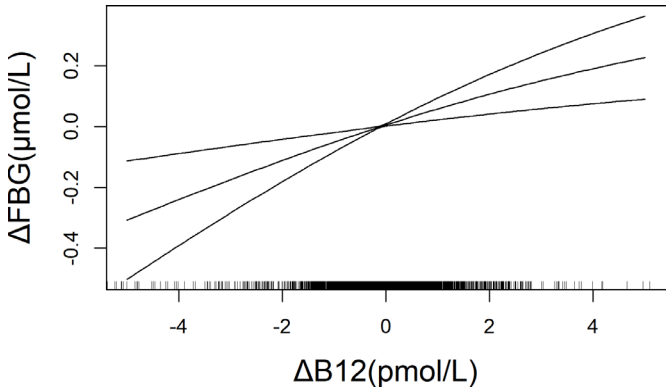

Figure 1 Multivariable-adjusted smoothing curves of change in vitamin B_{12} and change in FBG in a subsample that included a total of 4366 subjects with both baseline and exit FBG and vitamin B_{12} measurements. Adjusted for age, sex, *MTHFR* gene C677T polymorphisms, SBP and DBP at baseline, mean SBP and DBP during the treatment period, body mass index, study center, baseline serum concentrations of folate, homocysteine, FBG, vitamin B_{12}, total cholesterol, triglycerides, and high-density lipoprotein cholesterol, treatment group, smoking status, and alcohol consumption status. DBP, diastolic blood pressure; FBG, fasting blood glucose; *MTHFR*, methylenetetrahydrofolate reductase; SBP, systolic blood pressure.

Clinical implications of findings

The role of vitamin B_{12} in DM varied by patient characteristics. Most previous studies have shown that vitamin B_{12} supplementation is necessary in elderly patients with diabetes with low vitamin B_{12} levels or in patients with diabetes with long-term metformin use.[8 17 20] Our study, along with other longitudinal studies, however, does not support the routine use of vitamin B_{12} supplementation to reduce the risk of new-onset DM[18] in relatively young patients with no evidence of vitamin B_{12} deficiency. Moreover, a meta-analysis by Valdés-Ramos *et al*[21] indicated no recommendation for the use of vitamin supplements in patients with type 2 diabetes mellitus. Of note, the research of Looker *et al*[16] showed that vitamin B_{12} was positively associated with all-cause mortality and death from diabetes/nephropathy, and previous data also indicated that elevated serum vitamin B_{12} levels are a predictive factor for mortality in elderly patients with cancer.[22] Salles *et al*[23] and Hemmersbach-Miller *et al*[24] reported that higher vitamin B_{12} levels might also be a marker to assess a higher risk of mortality in elderly patients. Vitamin B_{12}

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can also accelerate decline in renal function and increase the risk of cardiovascular events in patients with impaired renal function.[25 26] Zeitlin *et al*[27] also suggest that for elderly people, vitamin B_{12} supplementation should not be routinely provided unless there are clear indications for doing so (a deficiency state), and then to only replace enough vitamin B_{12} to correct the deficiency. Through our research and analysis, we found that vitamin B_{12} may still have a correlation with blood glucose or DM, and the disappearance of this correlation in the longitudinal analysis may be due to the relative changes in the observation age, the decrease in vitamin B_{12} levels and the increase in FBG levels over time. Therefore, the relationship between the changes in indicators needs to be observed to reflect real results. We found that the change in vitamin B_{12} levels and the change in FBG levels showed a positive vitamin B_{12}–FBG association in the subsample. In addition, we repeated the previous analysis with this subsample and found the results were consistent with those of the previous analysis (online supplementary table S3).

The present study had some limitations. First, this study focused on Chinese adults with hypertension, so the generalizability of the results to other populations remains to be determined. Second, new-onset DM was not a primary outcome or a prespecified outcome of the CSPPT. We did not obtain FBG measurements at the scheduled follow-up visits, nor did we measure hemoglobin A1c or perform glucose tolerance tests at baseline or during the follow-up visits. Therefore, it is possible that we have underestimated the incidence of new-onset DM in the CSPPT. Nevertheless, we believe that any potential underestimation of new-onset DM should be non-differential, and therefore should not significantly affect the results. Finally, we only measured vitamin B_{12} levels on a small subset (n=4366) of the population at the exit visit and were unable to examine vitamin B_{12} dynamics during the follow-up period of the CSPPT.

CONCLUSION

Among a population of adults with hypertension in China without a history of stroke or MI, who were mostly vitamin B_{12}-sufficient, there was a dose–response association of vitamin B_{12} levels with the risk of DM based on cross-sectional analyses at baseline. There was no prospective relationship between baseline vitamin B_{12} and new-onset DM in the longitudinal analyses. However, in a subsample, a positive vitamin B_{12}–FBG association was shown by the change in vitamin B_{12} levels and the change in FBG levels. This result indicates that there may be a potential correlation between B_{12} and diabetes. Our findings illustrate a clear discrepancy in results from the cross-sectional and longitudinal analyses even from the same study population, and underscore the need to consider both baseline and longitudinal changes between vitamin B_{12} and FBG in order to better elucidate the role of vitamin B_{12} in the development of diabetes. If further studies confirm such findings, this will have an important impact on clinical and public health.

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REFERENCES

1 Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. *Lancet* 2017;389:2239–51.
2 Chinese Diabetes Society, National Offic for Primary Diabetes Care. [National guidelines for the prevention and control of diabetes in primary care(2018)]. *Zhonghua Nei Ke Za Zhi* 2018;57:885–93.
3 O'Leary F, Samman S. Vitamin B12 in health and disease. *Nutrients* 2010;2:299–316.
4 Yang W, Cai X, Wu H, *et al*. Associations between metformin use and vitamin B_{12} levels, anemia, and neuropathy in patients with diabetes: a meta-analysis. *J Diabetes* 2019;11:729–43.
5 Bauman WA, Shaw S, Jayatilleke E, *et al*. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. *Diabetes Care* 2000;23:1227–31.
6 Jayashri R, Venkatesan U, Rohan M, *et al*. Prevalence of vitamin B_{12} deficiency in South Indians with different grades of glucose tolerance. *Acta Diabetol* 2018;55:1283–93.
7 Song Y, Cook NR, Albert CM, *et al*. Effect of homocysteine-lowering treatment with folic acid and B vitamins on risk of type 2 diabetes in women: a randomized, controlled trial. *Diabetes* 2009;58:1921–8.
8 Reinstatler L, Qi YP, Williamson RS, *et al*. Association of biochemical B_{12} deficiency with metformin therapy and vitamin B_{12} supplements: the National health and nutrition examination survey, 1999-2006. *Diabetes Care* 2012;35:327–33.
9 Huo Y, Li J, Qin X, *et al*. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPT randomized clinical trial. *JAMA* 2015;313:1325–35.
10 Huang X, Li Y, Li P, *et al*. Association between percent decline in serum total homocysteine and risk of first stroke. *Neurology* 2017;89:2101–7.
11 Huang X, Qin X, Yang W, *et al*. *MTHFR* Gene and Serum Folate Interaction on Serum Homocysteine Lowering: Prospect for Precision Folic Acid Treatment. *Arterioscler Thromb Vasc Biol* 2018;38:679–85.
12 American Diabetes Association. 2. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes-2018*. *Diabetes Care* 2018;41:S13–27.
13 Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. *BMJ* 2014;349:g5226.
14 Moen G-H, Qvigstad E, Birkeland KI, *et al*. Are serum concentrations of vitamin B-12 causally related to cardiometabolic risk factors and disease? A Mendelian randomization study. *Am J Clin Nutr* 2018;108:398–404.
15 Margalit I, Cohen E, Goldberg E, *et al*. Vitamin B12 deficiency and the role of gender: a cross-sectional study of a large cohort. *Ann Nutr Metab* 2018;72:265–71.
16 Looker HC, Fagot-Campagna A, Gunter EW, *et al*. Homocysteine and vitamin B(12) concentrations and mortality rates in type 2 diabetes. *Diabetes Metab Res Rev* 2007;23:193–201.
17 Kwok T, Lee J, Ma RC, *et al*. A randomized placebo controlled trial of vitamin B_{12} supplementation to prevent cognitive decline in older diabetic people with borderline low serum vitamin B_{12}. *Clin Nutr* 2017;36:1509–15.
18 Rafnsson SB, Saravanan P, Bhopal RS, *et al*. Is a low blood level of vitamin B12 a cardiovascular and diabetes risk factor? A systematic review of cohort studies. *Eur J Nutr* 2011;50:97–106.
19 Eshak ES, Iso H, Muraki I, *et al*. Among the water-soluble vitamins, dietary intakes of vitamins C, B_2 and folate are associated with the reduced risk of diabetes in Japanese women but not men. *Br J Nutr* 2019;121:1357–64.
20 Gupta K, Jain A, Rohatgi A. An observational study of vitamin B12 levels and peripheral neuropathy profile in patients of diabetes mellitus on metformin therapy. *Diabetes Metab Syndr* 2018;12:51–8.
21 Valdés-Ramos R, Guadarrama-López AL, Martínez-Carrillo BE, *et al*. Vitamins and type 2 diabetes mellitus. *Endocr Metab Immune Disord Drug Targets* 2015;15:54–63.
22 Geissbühler P, Mermillod B, Rapin CH. Elevated serum vitamin B12 levels associated with CRP as a predictive factor of mortality in palliative care cancer patients: a prospective study over five years. *J Pain Symptom Manage* 2000;20:93–103.
23 Salles N, Herrmann F, Sakbani K, *et al*. High vitamin B12 level: a strong predictor of mortality in elderly inpatients. *J Am Geriatr Soc* 2005;53:917–8.
24 Hemmersbach-Miller M, Conde-Martel A, Betancor-León P. Vitamin B as a predictor of mortality in elderly patients. *J Am Geriatr Soc* 2005;53:2035–6.
25 Spence JD, Yi Q, Hankey GJ. B vitamins in stroke prevention: time to reconsider. *Lancet Neurol* 2017;16:750–60.
26 House AA, Eliasziw M, Cattran DC, *et al*. Effect of B-vitamin therapy on progression of diabetic nephropathy: a randomized controlled trial. *JAMA* 2010;303:1603–9.
27 Zeitlin A, Frishman WH, Chang CJ. The association of vitamin B 12 and folate blood levels with mortality and cardiovascular morbidity incidence in the old old: the Bronx aging study. *Am J Ther* 1997;4:275–81.

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