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Vitamin B and One-Carbon Metabolite Profiles Show Divergent Associations with Cardiometabolic Risk Markers but not Cognitive Function in Older New Zealand Adults: A Secondary Analysis of the REACH Study

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A B S T R A C T

Background: Vitamin B inadequacies and elevated homocysteine status have been associated with impaired cognitive and cardiometabolic health with aging. There is, however, a scarcity of research investigating integrated profiles of one-carbon (1C) metabolites in this context, including metabolites of interconnected folate, methionine, choline oxidation, and transsulfuration pathways.
Objectives: The study aimed to examine associations between vitamins B and 1C metabolites with cardiometabolic health and cognitive function in healthy older adults, including the interactive effects of Apolipoprotein E-ε4 status.
Methods: Three hundred and thirteen healthy participants (65-74 y, 65% female) were analyzed. Vitamins B were estimated according to dietary intake (4-d food records) and biochemical status (serum folate and vitamin B_{12}). Fasting plasma 1C metabolites were quantified by liquid chromatography with tandem mass spectrometry. Measures of cardiometabolic health included biochemical (lipid panel, blood glucose) and anthropometric markers. Cognitive function was assessed by the Computerized Mental Performance Assessment System (COMPASS) and Montreal Cognitive Assessment (MoCA). Associations were analyzed using multivariate linear (COMPASS, cardiometabolic health) and Poisson (MoCA) regression modeling.
Results: Over 90% of participants met dietary recommendations for riboflavin and vitamins B_6 and B_{12}, but only 78% of males and 67% of females achieved adequate folate intakes. Higher serum folate and plasma betaine and glycine concentrations were associated with favorable cardiometabolic markers, whereas higher plasma choline and homocysteine concentrations were associated with greater cardiometabolic risk based on body mass index and serum lipids concentration values ($P < 0.05$). Vitamins B and homocysteine were not associated with cognitive performance in this cohort, though higher glycine concentrations were associated with better global cognitive performance ($P = 0.017$), episodic memory ($P = 0.016$), and spatial memory ($P = 0.027$) scores. Apolipoprotein E-ε4 status did not modify the relationship between vitamins B or 1C metabolites with cognitive function in linear regression analyses.
Conclusions: Vitamin B and 1C metabolite profiles showed divergent associations with cardiometabolic risk markers and limited associations with cognitive performance in this cohort of healthy older adults.

Keywords: One-carbon metabolites, older adults, vitamins B, cognition, homocysteine, choline, glycine, metabolic health

Abbreviations: 1C, one-carbon; *APOE*, apolipoprotein E; COMPASS, Computerized Mental Performance Assessment System; DMG, dimethylglycine; EAR, estimated average requirement; LC-MS/MS, Liquid chromatography coupled with tandem mass spectrometry; MoCA, Montreal Cognitive Assessment.

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Introduction

Gradual cognitive decline is considered part of the normal aging process, which can progress to mild cognitive impairment when the decline exceeds that expected for an individual's age and education level [1]. Half of all individuals with mild cognitive impairment are expected to develop dementia within 5 y of diagnosis [1], prompting the development of strategies to prevent or delay the onset of mild cognitive impairment to become a major public health priority.

One's diet contributes to the maintenance or deterioration of cognition, influenced by broader dietary patterns through to individual nutrients [2]. Vitamins B have a long-standing relationship with cognitive health, attributed to their role in regulating one-carbon (1C) metabolism [3]. Here, vitamins B are required for several processes related to cognition, including DNA synthesis and repair, methylation reactions, and homocysteine regulation. Over 2 decades ago, seminal reports showed elevated circulating homocysteine and low folate or vitamin B_{12} status in patients with dementia compared with healthy controls according to both clinical and histopathological criteria [4,5]. Following this, a wealth of epidemiological data ensued to support the association between cognitive function and vitamin B or homocysteine status in both cross-sectional and prospective settings [6,7, 8–17]. Paradoxically, very high folate intake or status has more recently been associated with poor cognitive function or accelerated cognitive decline in older adults, particularly in those with suboptimal vitamin B_{12} intake [18–20]. Understanding of this long-standing relationship between vitamins B and cognition in the literature is by no means complete.

The folate, methionine, choline oxidation, and transsulfuration pathways intersect at homocysteine in 1C metabolism (Figure 1). Homocysteine is considered an overarching marker of 1C metabolism and correspondingly has been the focus of vitamins B and 1C metabolism research. Although evidence relating to transsulfuration metabolites (cysteine, cystathionine, serine) is limited, literature suggests involvement of choline oxidation metabolites (choline, betaine, dimethylglycine [DMG]) in cognitive function. In particular, higher dietary choline intake [21,22] and status [23] are associated with improved cognitive performance and protection against cognitive decline in older adults. Higher betaine concentrations have also been associated with memory performance in older adults [24], and greater increases in plasma DMG concentrations were associated with improved memory performance in a 24-wk vitamin supplementation study [24]. Although homocysteine is an established clinical biomarker, relying on a single marker is a simple interpretation of this complex pathway, which possibly impedes a more discerning understanding of the association between 1C nutrients and cognitive health outcomes.

1C metabolites demonstrate this complexity through divergent associations with cardiometabolic health [25–29]. Since metabolic dysregulation accelerates cognitive aging [30–33], the intersection of 1C metabolism with cognitive and cardiometabolic health should be considered in disentangling the vitamin B-cognition relationship. Of relevance here is Apolipoprotein E, a plasma glycoprotein involved in lipoprotein metabolism and neuronal repair [34]. Carrying the Apolipoprotein E(*APOE*)-ε4 allele is a risk factor for developing cardiovascular disease [35] and Alzheimer's disease [34]. Although the role of *APOE*-ε4 in modifying risk for cognitive decline is somewhat contentious [36], emerging evidence indicates an interactive effect of *APOE*-ε4 status with vitamin B status or homocysteine in modifying cognitive impairment [37–40].

This study aimed to investigate the association of vitamins B and 1C metabolites with cognition and cardiometabolic health, including the interactive effects of *APOE*-ε4 status on cognition in healthy older adults.

Methods

Study design

The current study reports on data from the Researching Eating, Activity, and Cognitive Health (REACH) study. This was a single-center, cross-sectional study conducted between April 2018 and February 2019, with details of study design and data collection available in the published study protocol [41]. The primary outcome relating to dietary patterns and cognitive performance has been published elsewhere [42], as has a report of associations between dietary patterns and metabolic syndrome [43]. This secondary analysis of the REACH cohort reports vitamins B and 1C metabolites alongside cognitive and cardiometabolic parameters. This analysis is reported according to the STROBE statement (Supplementary File 2).

The participants signed written informed consent at the research facility, and this study was approved by the Massey University Human Ethics Committee: Southern A, Application 17/69.

Participants

Three hundred seventy-one males and females aged 65 to 74 y living independently in the community in Auckland, New Zealand, were recruited to participate in the REACH cohort. Participants were excluded if they reported a diagnosis of dementia or other conditions that may impair cognitive function (stroke, traumatic head or brain injury, or a neurological or psychiatric disorder), were taking medication that may influence cognitive function, were color blind (color recognition was required for cognitive testing), or not proficient in English. Participants were further excluded if they experienced an event in the last 2 y which substantially impacted dietary intake or cognitive function (e.g., death of a family member) or if another household member was enrolled in the study.

Cognitive testing

The Montreal Cognitive Assessment (MoCA) test was administered by trained examiners to assess global cognitive performance, with a score of <26 defined as mild cognitive impairment [44]. The Computerized Mental Performance Assessment System (COMPASS; Northumbria University, Newcastle upon Tyne, UK) was used to examine global cognition and multiple cognitive domains. The COMPASS cognitive battery measures participants' attention (and vigilance), executive function, episodic memory, working memory, and spatial memory. The tests used to assess these domains are outlined in Table 1 and have been reported in full in previous publications of the REACH study [42].

Cognitive testing with COMPASS took approximately 1 h, with the first 15 min comprised of a training exercise and a 5-min break before the actual assessment began. Testing was

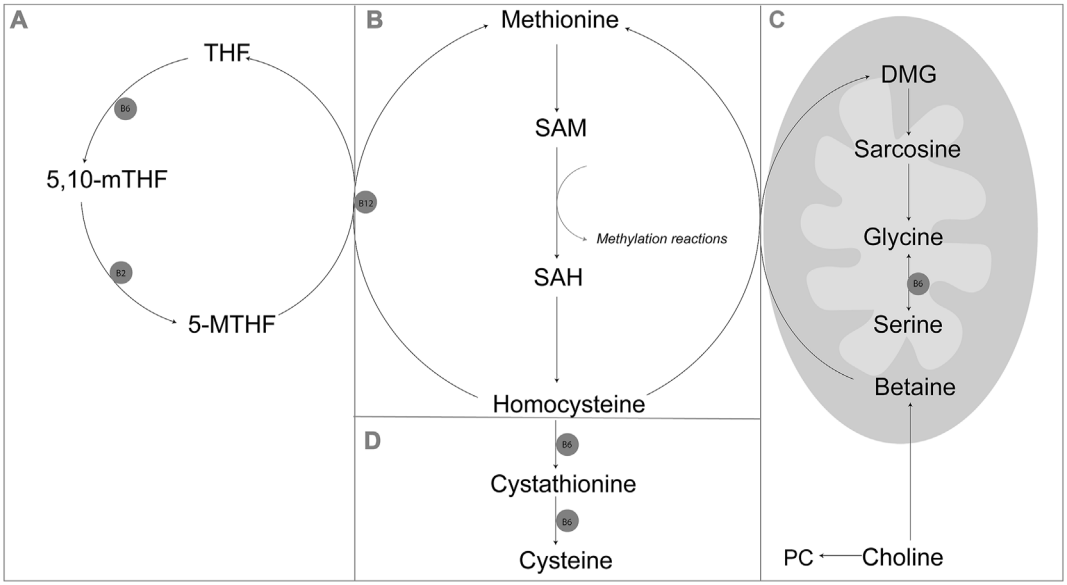

FIGURE 1. Schematic overview of the pathways comprising one-carbon metabolism.
The A) Folate cycle, B) Methionine cycle, C) Choline oxidation pathway (predominantly occurring in the mitochondria), and D) Transsulfuration pathway intersect at homocysteine in one-carbon metabolism. Vitamins acting as coenzymes are presented in a grey circle.

completed in a controlled environment (noise and temperature) at the same time of day (morning), and after the provision of a standard breakfast to minimize any effects, food may have on cognition [45]. Participants were instructed to avoid undue stress, alcohol, recreational drugs, and nonroutine physical activity prior to their study visit. Test scores were cleaned (e.g., removing a test that had been restarted due to the participant requiring a break), and then a z-score was created (within the REACH cohort).

Dietary intake and analysis

Dietary intake was measured from 4-d estimated food records, which spanned 4 consecutive days and included at least one weekend day. During their study visit, participants were informed of the need to record all food and beverages consumed accurately through an instructional video and had the opportunity to resolve questions with research staff.

Food records were completed within 1 mo of participants' study visit and sent to the REACH study coordinator. Food records were then checked for completion prior to analysis, and participants were contacted if further detail was required. Dietary analysis was completed by 4 trained Nutritionists and Dietitians using Foodworks (Version 10; Xyris Software, Australia), which utilizes Food Composition Databases from New Zealand (FOODfiles 2016, Version 01) and Australia (AusFoods 2019, AusBrands 2019). Consistency of entries was achieved by using a register of common food items during data entry, which was checked once all food records were entered. Finally, one Dietitian checked every food record entered for accuracy and consistency. Following final data inspection, the plausibility of reported energy intake was compared with cut-off values specific for older adults. Food records were excluded if daily energy intake was $<$ 2100 kJ (500 kcal) or $>$14,700 kJ (3500 kcal) for omen, and $<$3350 kJ (800 kcal) or $>$16,800 kJ (4000 kcal) for males [46,47]. Nutrient intake was compared with age- and sex-specific Estimated Average Requirements (EAR) according to the joint Australia and New Zealand Nutrient Reference Values [48].

The New Zealand food supply includes foods fortified with folic acid and other B vitamins on a voluntary basis at the time of this study. Both natural and fortified food sources were accounted for in the food records and entered into the FOODfiles

TABLE 1
Summary of tests used and cognitive domains measured from the Computerized Mental Performance Assessment System (COMPASS) battery of cognitive assessments

Cognitive domain	Cognitive domain description	Tests
Attention	• Attention: Ability to concentrate on selected environmental aspects while ignoring others • Vigilance: Ability to maintain attention and alertness over time	• Simple reaction time • Choice reaction time • Digit vigilance task
• Episodic memory	• Ability to retain memories that can be consciously recorded	• Immediate and delayed word recall • Delayed word recognition • Delayed picture recognition
• Executive function	• Ability to co-ordinate cognitive responses	• Stroop test
• Spatial memory	• Ability to co-ordinate visuo-spatial memory	• Computerized location learning • Computerized location recall
• Working memory	• Ability to retain information while carrying out more complex cognitive tasks	• Corsi blocks
• Global performance	• Average performance across all cognitive domains	• Average performance across all tests

2016 food composition database, and the research team probed for detailed information regarding commonly fortified foods (e.g., cereals, breads) or beverages (e.g., drink powders). Dietary intake does not include supplemental sources of vitamins B due to participants reporting 'occasional' supplement use, and with uncertain frequency of intake, we cannot reliably confirm the contribution of supplemental vitamins B to dietary adequacy.

Biochemical analysis

Following an overnight fast of at least 9 h, a qualified phlebotomist collected blood samples into serum and EDTA-coated vacutainers. Plasma samples were placed on ice, spun within 2 h of collection, and centrifuged (Heraeus Labofuge 400R) for 15 min at 3500 × *g* (1547 g-force) at 4°C. For serum samples, whole blood was allowed to clot for 30 min, placed on ice, and centrifuged as per above. Blood glucose and lipid profiles were measured on the study day using point-of-care systems. All other samples were separated into aliquots, stored in Eppendorf tubes at -80°C, and thawed immediately before analysis.

One-carbon metabolites and vitamins B

Plasma concentrations of 1C metabolites (betaine, choline, cystathionine, cysteine, DMG, homocysteine, methionine, *S*-adenosylhomocysteine, and *S*-adenosylmethionine) were quantified by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), following previously reported methods [49]. Briefly, an automated robotic liquid handling system (Eppendorf epMotion 5075vt) was used to prepare plasma samples. 300µl of 1% formic acid in methanol was pipetted into a 96-well IMPACT protein precipitation plate (Phenomenex, Torrance). All standards (100µL), quality controls (100µL), and samples (100µL) were spiked with 20µL of internal standard solution, agitated for 5 min on the robot's thermomixer (room temperature, 800 × *g*), then filtered into a 96-well collection plate (Phenomenex, Torrance) by applying a vacuum (450 mbar). Tris (2-carboxyethyl) phosphine (100µL) was then dispensed into each well, which reduces disulfide bonds in cystine and homocysteine to allow for the separate quantification of cysteine and homocysteine, respectively.

Three sets of quality control samples were included to assess the recovery of standards and reproducibility of samples. Metabolites were considered acceptable if standard recoveries were between 80% and 120% and coefficients of variance were < 20%. Cystathionine and *S*-adenosylhomocysteine were excluded from further analysis because they did not satisfy coefficients of variance requirements. Homocysteine samples from one batch also required exclusion, and missing value imputation was not performed as this was a failing of the whole plate (22%; $n = 70$).

A Cobas e411 autoanalyzer (Roche) was used to measure serum vitamin B_{12} by electrochemiluminescence immunoassay and serum folate by a binding assay.

Cardiometabolic markers

Fasting blood glucose was measured by a HemoCue Glucose 201 RT System (Radiometer Pacific Pty. Ltd.). A Cobas b 101 system (Roche) was used to measure lipid profile values (total cholesterol, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, and triglycerides concentration).

Apolipoprotein E genotype

The methods for measuring presence of the *APOE*-ε4 allele have been reported elsewhere [42]. In brief, this was measured at an accredited laboratory (Grafton Clinical Genomics) using the DSP DNA Mini Kit for DNA extraction, with *APOE* ε2, ε3, ε4 (SNP ID rs7412 and rs429358) alleles detected using the Agena MassARRAY system [50].

Anthropometric data and blood pressure

Weight was measured to the nearest 0.01 kg (Tanita Electronic Scales), and height was measured to the nearest 0.1 cm using a portable stadiometer. Waist and hip circumference were measured with a flexible steel tape (Lufkin W600 PM) following standard methods outlined by the International Society for the Advancement of Kinanthropometry (ISAK) [51].

Blood pressure was measured after participants were seated and resting quietly for 5 min. The average systolic and diastolic blood pressure was calculated from 2 measurements taken by a digital automatic blood pressure monitor (Omron HEM-907), with a 1-min rest period between measures.

Metabolic syndrome

According to the American Heart Association/National Health, Lung and Blood Institute criteria [52], metabolic syndrome was defined if any 3 of the following 5 measures were met or medication was used to manage: elevated waist circumference (≥ 102cm in males, ≥ 88cm in females), elevated triglycerides concentration (≥ 1.7 mmol/L), reduced HDL-C concentration (< 1.03 mmol/L in males, < 1.3 mmol/L in females), elevated blood pressure (≥ 130 mm Hg systolic, or ≥ 85 mm Hg diastolic), or elevated fasting glucose concentration (≥ 5.6 mmol/L).

Sociodemographic, health, and physical activity data

Sociodemographic and health information were collected through written questionnaires, which were completed in person and checked for completeness and sensibility. Variables included in this secondary analysis were age, sex, ethnicity, socioeconomic status, education (highest level achieved), physical activity, history of anxiety or depression, medication (polypharmacy defined as ≥5 medications used [53]), and supplement use (vitamin B or multivitamin supplements).

Socioeconomic status was defined by the New Zealand Index of Multiple Deprivation according to residential address [54]. Physical activity was assessed by the International Physical Activity Questionnaire—short form [55] and categorized according to tertiles of physical activity.

Statistical analysis

Sample size estimates for the REACH study were calculated for the primary outcome of a linear association between cognitive performance and dietary patterns, leading to a sample estimate of 346 participants required to see a small effect size (Pearson correlation of 0.15) with 80% power [41]. This is likely adequate for 1C metabolites reported here based on the small to moderate effect size of associations reported with cognitive performance [24] and cardiometabolic health [56] in older adults.

Table 2
Study population characteristics for the subset of participants in the REACH cohort included in this analysis

• Participant characteristics	Total population	Males	Females	*P*
n	• 313	• 111 (35)	• 202 (65)	
• Demographics				
• Age	• 69.8 (2.6)	• 70.3 (2.5)	• 69.5 (2.6)	• 0.019*
• Highest education achieved, *n* (%)				• 0.011*
No qualification	5 (1.6)	1 (1.0)	4 (2.0)	
Secondary school	66 (21)	15 (14)	51 (25)	
Post-secondary school	127 (41)	42 (38)	85 (42)	
University	115 (37)	53 (48)	62 (31)	
• Lifestyle				
• Polypharmacy, *n* (%)	• 25 (8.0)	• 11 (9.9)	• 14 (6.9)	• 0.476
• Supplement use[1], *n* (%)	• 53 (17)	• 18 (16)	• 35 (17)	• 0.926
• Vitamin B status				
• Serum folate, nmol/L	• 27.9 (10.8)	• 26.4 (9.9)	• 28.8 (11.2)	• 0.059
• Serum vitamin B_{12}, pmol/L	• 373 (147)	• 352 (121)	• 386 (159)	• 0.040*
• Dietary intake				
• Energy, kJ/day	• 8095 (1918)	• 9378 (1956)	• 7463 (1510)	• <0.001*
• Folate, μg/day	• 429 (184)	• 468 (194)	• 412 (174)	• 0.012*
• Riboflavin, mg/day	• 2.13 (0.79)	• 2.42 (0.87)	• 1.98 (0.70)	• <0.001*
• Vitamin B_6, mg/day	• 2.53 (0.89)	• 2.78 (1.00)	• 2.40 (0.79)	• <0.001*
• Vitamin B_{12}, μg/day	• 4.17 (3.60)	• 4.67 (3.54)	• 3.95 (3.73)	• 0.089
• Dietary adequacy[2]				
• Folate, *n* (%)	• 222 (71)	• 87 (78)	• 135 (67)	• 0.052
• Riboflavin, *n* (%)	• 303 (97)	• 107 (96)	• 196 (97)	• 0.925
• Vitamin B_6, *n* (%)	• 303 (97)	• 109 (98)	• 194 (96)	• 0.647
• Vitamin B_{12}, *n* (%)	• 290 (93)	• 105 (95)	• 185 (92)	• 0.549
• Cardiometabolic health				
• BMI, kg/m^2	• 26.0 (4.29)	• 26.6 (4.5)	• 25.6 (4.5)	• 0.065
• Glucose, mmol/L	• 4.40 (0.73)	• 4.50 (0.81)	• 4.35 (0.68)	• 0.093
• Serum HDL-cholesterol, mmol/L	• 1.62 (0.40)	• 1.43 (0.35)	• 1.73 (0.40)	• <0.001*
• Serum LDL-cholesterol, mmol/L	• 2.97 (0.94)	• 2.63 (0.95)	• 3.16 (0.88)	• <0.001*
• Total/HDL-cholesterol	• 3.36 (1.11)	• 3.40 (1.18)	• 3.34 (1.07)	• 0.663
• Serum triglycerides, mmol/L	• 1.25 (0.54)	• 1.26 (0.59)	• 1.23 (0.52)	• 0.605
• Systolic blood pressure, mm/Hg	• 139 (17.9)	• 140 (14.5)	• 139 (19.6)	• 0.390
• Diastolic blood pressure, mm/Hg	• 78.4 (10.4)	• 78.2 (11.1)	• 78.9 (9.10)	• 0.547
• Metabolic syndrome, *n* (%)	• 34 (11)	• 10 (9.0)	• 24 (12)	• 0.554
• Cognitive risk and status				
• ApoE-ε4 genotype, *n* (%)	• 82 (26)	• 27(24)	• 55 (27)	• 0.649
• History of anxiety/depression, *n* (%)	• 62 (20)	• 17 (15)	• 45 (22)	• 0.183
• Family history, *n* (%)	• 94 (30)	• 24 (22)	• 70 (35)	• 0.019*
• Prevalence of MCI, *n* (%)	• 93 (30)	• 34 (31)	• 59 (29)	• 0.842
• Metabolites (plasma)				
• Betaine, μM	• 34.1 (9.59)	• 38.8 (10.0)	• 31.5 (8.3)	• <0.001*
• Choline, μM	• 8.57 (2.19)	• 9.42 (2.21)	• 8.10 (2.04)	• <0.001*
• Cysteine, μM	• 98.7 (14.9)	• 104 (14.2)	• 96.2 (14.6)	• <0.001*
• Dimethylglycine, μM	• 2.14 (0.82)	• 2.39 (0.84)	• 2.01 (0.77)	• <0.001*
• Glycine, μM	• 209 (57.3)	• 183 (41.5)	• 223 (60.0)	• <0.001*
• Homocysteine[3], μM	• 2.26 (0.81)	• 2.57 (0.96)	• 2.10 (0.68)	• <0.001*
• Methionine, μM	• 21.2 (3.33)	• 22.5 (3.17)	• 20.4 (3.18)	• <0.001*
• SAM, μM	• 138 (65.5)	• 149 (62.9)	• 132 (66.4)	• <0.001*
• Serine, μM	• 79.6 (15.6)	• 75.2 (15.2)	• 82.0 (15.3)	• <0.001*

Data presented as mean (SD) for continuous variables or count (%) for categorical data. Differences in participant characteristics according to sex are presented according to *t*-test and chi-squared tests for continuous and categorical data, respectively
Abbreviations: MCI, mild cognitive impairment; SAM, *S*-adenosylmethionine
* indicating a significant difference ($P < 0.05$).
[1] Supplement use refers to individual (e.g., folic acid) or combined B vitamin supplements, or a multivitamin/mineral supplement.
[2] Dietary adequacy is defined as the number (%) of participants meeting the daily estimated average requirement of folate (320μg), riboflavin (males: 1.3mg, females: 1.1mg) vitamins B_6 (males: 1.4mg, females: 1.3mg) and B_{12} (2.0μg) according to the Australia and New Zealand Nutrient Reference Values(48). Dietary adequacy is based on intake from natural and fortified food sources, but does not include supplemental intakes.
[3] Homocysteine concentrations cannot be compared to expected reference ranges (≥15 μM is typically considered elevated (93)), as concentrations of the disulfide-bond reducing agent used in this analytical technique is lower than in other published methods (94). Smaller sample size (n = 243) available for analyses involving homocysteine due to one batch in the liquid chromatography mass spectrometry analysis not satisfying the coefficient of variance requirements.

Distribution was graphically assessed prior to analysis. DMG, glycine, homocysteine, and vitamin B_{12} (intake and serum concentrations) were logarithmically transformed to achieve approximately normal distribution. All statistical analyses were performed using R 3.6.3 statistical software [57]. Unless otherwise specified, $P < 0.05$ was considered significant, and data presented as mean and standard deviation.

Baseline participant characteristics were compared between males and females by *t*-test or chi-square test for continuous and categorical variables, respectively.

Associations between vitamin B intake, status, and 1C metabolite concentrations with cardiometabolic and cognitive outcomes were explored using multivariate linear regression (cardiometabolic parameters, COMPASS scores) and generalized linear modeling (Poisson family risk of mild cognitive impairment according to MoCA scores) analyses.

Model 1 refers to a "base" model adjusted for age, sex, and analytical batch effects (if including 1C metabolites). Model 2 was further adjusted for covariates associated with either cognitive or cardiometabolic parameters ($P < 0.150$ according to univariate analyses). This included energy intake, physical activity, polypharmacy and supplement (individual or combined vitamins B, or multivitamins) use for cardiometabolic outcomes, and energy intake, physical activity, supplement (individual or combined vitamins B, or multivitamins) use, and history of anxiety/depression for cognitive outcomes. A sensitivity analysis was performed by excluding participants who used supplements (individual or combined B vitamins, or multivitamins),and repeating the models to test the association between vitamin B intake, status, and 1C metabolites with cognitive performance (linear regression) or risk of mild cognitive impairment (generalized linear models, Poisson family). Because of the number of regression models tested, we will subsequently only discuss vitamins or metabolites that demonstrate more consistent associations (impacting at least 3 cardiometabolic or cognitive domains).

The impact of *APOE*-ε4 status (presence of *APOE*-ε4 allele—yes/no) on the relationship between vitamins B or 1C metabolites and cognitive performance in COMPASS testing was assessed in separate linear regression models. These models were fitted with an interaction term between *APOE*-ε4 status and vitamin B or 1C metabolites and were fully adjusted for covariates described in Model 2 above. The Tukey adjustment was applied to correct for multiple comparisons for any significant interaction term found.

Results

Participant characteristics

Of the 371 participants who completed the REACH study, 313 (69.8 ± 2.6 y, 65% female) were available for this secondary analysis (Table 2). Participants were excluded due to previous stroke or head injury which was not detected at screening ($n = 7$), or missing data for cognitive performance ($n = 2$), food records ($n = 38$), or blood samples available for 1C metabolite and vitamin B analysis ($n = 11$). A further 70 samples were excluded from analyses, including homocysteine, due to a failed LC-MS/MS batch, but these samples were included in other analyses. Differences in participant characteristics between included and excluded

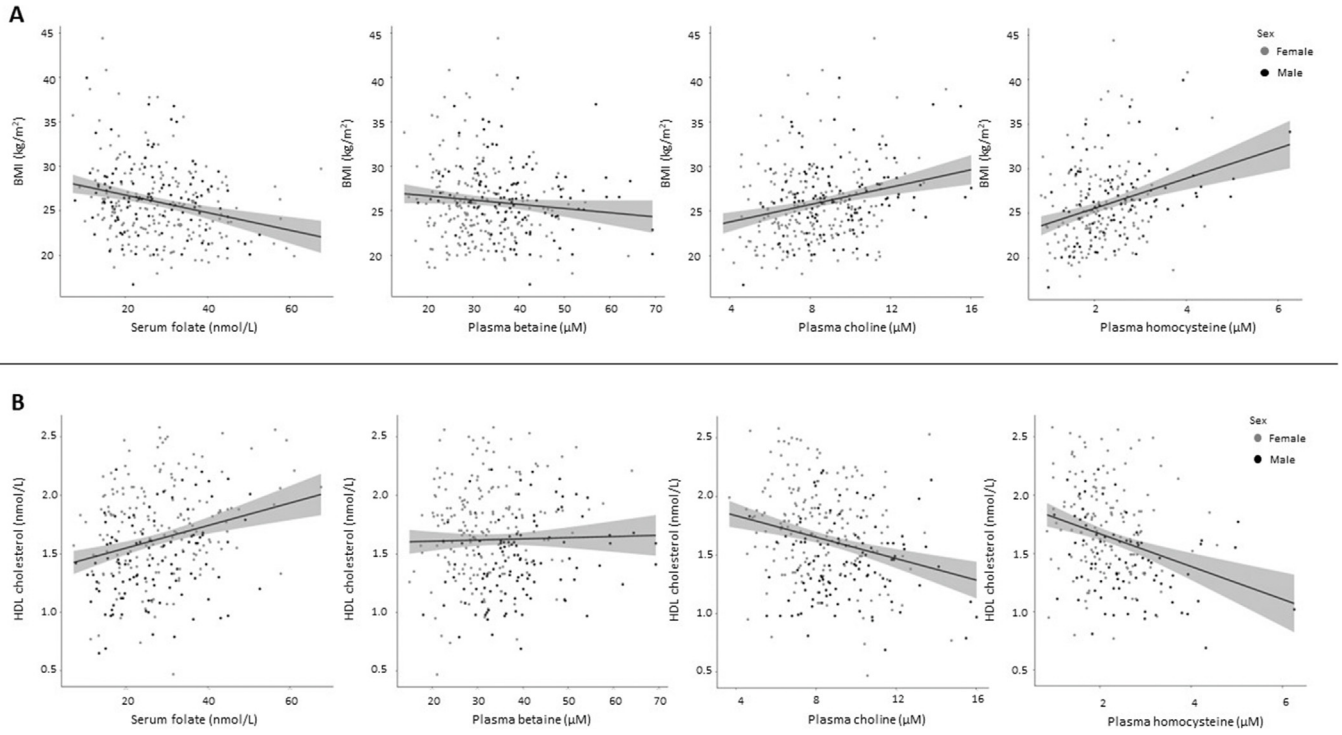

FIGURE 2. Associations between B vitamins and one-carbon metabolites with BMI and HDL-cholesterol.
A) Data is presented for significant associations found between BMI and serum folate (β=-0.177, $P = 0.001$), betaine (β=-0.071, $P = 0.009$), choline (β=0.509, $P < 0.001$), and homocysteine (β=8.016, $P < 0.001$ in log-transformed analyses) in fully adjusted regression models. B) Data is presented for significant associations found between HDL-cholesterol and serum folate (β=0.016, $P = 0.001$), betaine (β=0.008, $P = 0.001$), choline (β=-0.028, $P = 0.014$), and homocysteine (β=-0.470, $P = 0.006$ in log-transformed analyses) in fully adjusted regression models. Corresponding data is presented in Supplemental Table 2.

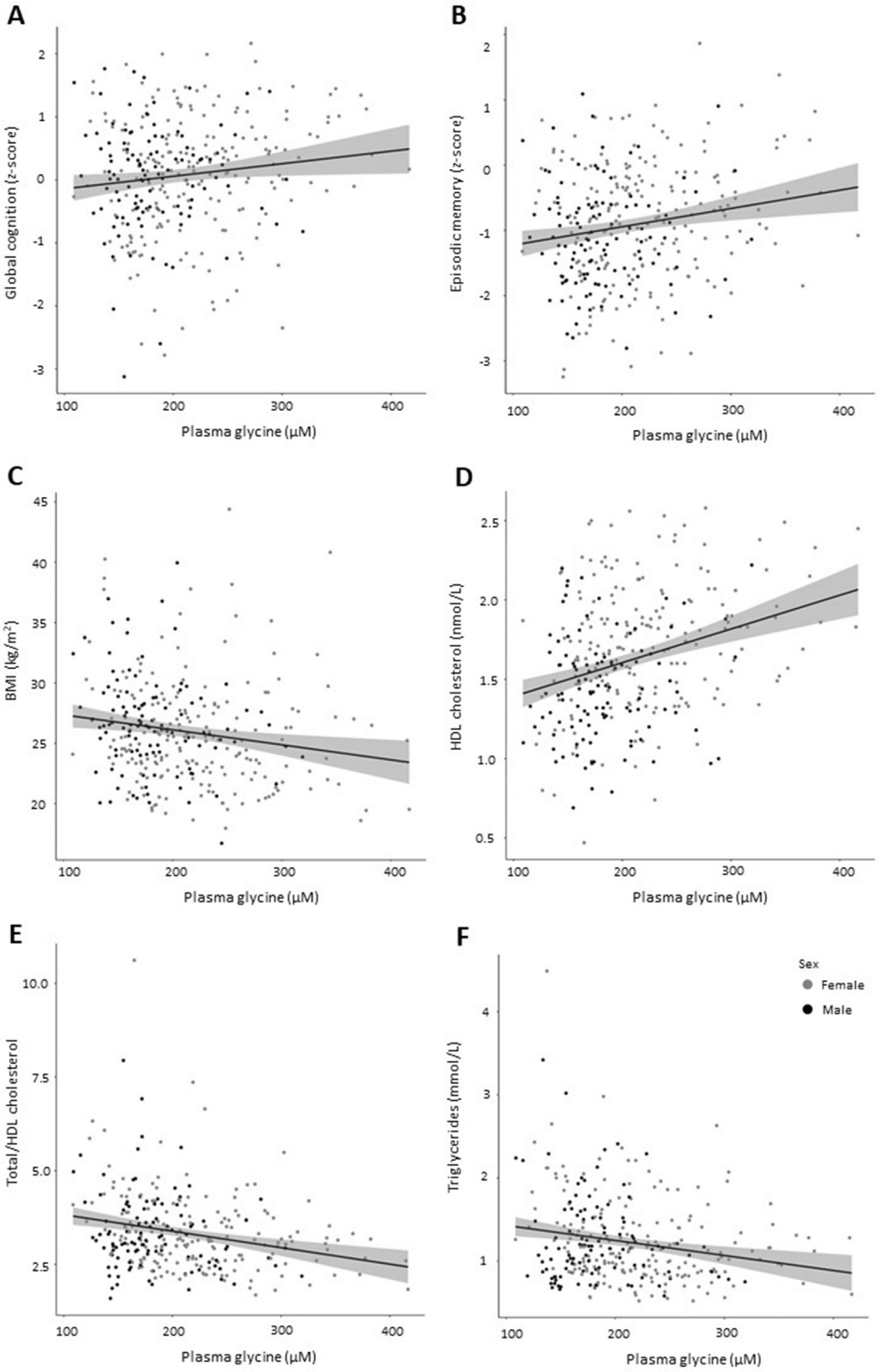

FIGURE 3. Association between glycine concentrations and measures of cognition and cardiometabolic health.
Data is presented for significant associations found between log-transformed glycine concentrations and A) global cognition (β=1.340, P =0.017), B) episodic memory (β=1.396, P = 0.016), C) BMI (β=-7.987, P = 0.002), D) HDL cholesterol (β=0.701, P = 0.003), E) Total/HDL-cholesterol ratio (β=-2.191, P < 0.001), and F) triglycerides (β=-1.204, P < 0.001) in fully adjusted regression models. Corresponding data is presented in Supplemental Tables 2 and 3.

TABLE 3
Vitamins B and one-carbon metabolites as predictors of mild cognitive impairment according to generalized linear (Poisson) models

	Model 1			Model 2		
	• Risk ratio	• 95% CI	• *P*	Risk ratio	95% CI	*P*
Folate intake	• 0.999	• 0.99, 1.00	• 0.783	• 1.000	• 0.99. 1.00	• 0.776
• Riboflavin intake	• 1.076	• 0.86, 1.33	• 0.520	• 1.204	• 0.93, 1.53	• 0.149
• Vitamin B_6 intake	• 1.177	• 0.97, 1.42	• 0.099	• 1.295	• 1.04, 1.60	• 0.020
• Vitamin B_{12} intake[1]	• 1.019	• 0.97, 1.06	• 0.378	• 1.021	• 0.97, 1.06	• 0.342
• Serum Folate	• 1.003	• 0.97, 1.04	• 0.864	• 1.001	• 0.96, 1.04	• 0.970
• Serum Vitamin B_{12}[1]	• 1.000	• 1.00, 1.00	• 0.759	• 1.00	• 1.00, 1.00	• 0.913
• Plasma Betaine	• 0.990	• 0.97, 1.01	• 0.347	• 0.989	• 0.97, 1.01	• 0.327
• Plasma Choline	• 0.952	• 0.88, 1.06	• 0.454	• 0.966	• 0.88, 1.06	• 0.470
• Plasma Cysteine	• 0.996	• 0.98, 1.01	• 0.614	• 0.998	• 0.98, 1.01	• 0.814
• Plasma DMG[1]	• 0.484	• 0.15, 1.58	• 0.235	• 0.473	• 0.14, 1.58	• 0.229
• Plasma Glycine[1]	• 0.665	• 0.09, 4.60	• 0.682	• 0.486	• 0.06, 3.69	• 0.490
• Plasma Homocysteine[1]	• 0.684	• 0.16, 2.01	• 0.607	• 0.787	• 0.18, 3.48	• 0.751
• Plasma Methionine	• 0.999	• 0.94, 1.05	• 0.959	• 0.998	• 0.94, 1.05	• 0.936
• Plasma SAM	• 1.000	• 1.00, 1.00	• 0.758	• 1.00	• 0.99, 1.00	• 0.786
• Plasma Serine	• 0.990	• 0.98, 1.00	• 0.136	• 0.988	• 0.97, 1.00	• 0.081

Risk ratios, 95% confidence intervals and *P* values presented for each metabolite fit in generalized linear (Poisson family) models. Mild cognitive impairment was defined as a MoCA test score of < 26. Model 1 is adjusted for age, sex, education, and batch effect. Model 2 was further adjusted for energy intake, physical activity, history of anxiety/depression, and supplement use (B vitamin or multivitamin and mineral).
*Indicates a significant association ($P < 0.05$).
[1] Fit as log-transformed variables in models. Abbreviations: DMG, dimethylglycine; SAM, *S*-adeonsylmethionine.

participants are highlighted in Supplementary Table 1. Briefly, participants included in this secondary analysis were marginally older and had a different make-up of education qualifications compared with the total REACH cohort, but there were no differences in participant characteristics for those who were excluded from homocysteine analyses.

Vitamin B intake and status

The majority of REACH participants met the EAR for intakes of riboflavin (males, 96%; females, 97%), vitamins B_6 (males, 98%; females, 96%), B_{12} (males, 95%; females, 92%). However, there was a relatively high prevalence of folate inadequacy, only 78% of males and 67% of females (sex difference, $P = 0.052$) meeting the EAR for folate of 320 μg/d (Table 2). On average, males had higher intakes of folate ($P = 0.012$), riboflavin ($P < 0.001$), and vitamin B_6 ($P < 0.001$) but similar vitamin B_{12} ($P = 0.089$) intake to females. Females had higher serum vitamin B_{12} concentrations than males (females: 386 ± 159, males:352 ± 121 pmol/L, $P = 0.040$), though differences in serum folate concentrations did not reach significance (females: 28.8 ± 11.2, males:26.4 ± 9.9 nmol/L, $P = 0.059$). Mean concentrations were well above deficiency cut-offs for serum folate (>10 nmol/L) and vitamin B_{12} (>150 pmol/L) [58]. Markers of cardiometabolic health and prevalence of mild cognitive impairment were largely similar, although females had higher concentrations of HDL- and LDL-cholesterol ($P < 0.001$) than males. Twenty-six percent of participants carried at least one *APOE*-ε4 allele, though this did not differ between males and females.

Sex differences in cardiometabolic markers and one-carbon metabolites

Similar to vitamin B intake and status, we see clear sex differences in cardiometabolic and 1C metabolite status in this cohort of older adults. This includes lower serum concentrations of betaine, choline, cysteine, DMG, homocysteine, methionine, and *S*-adenosylmethionine but higher glycine and serine concentrations ($P < 0.001$) in females. Females also have higher HDL- and LDL-cholesterol status compared with males ($P < 0.001$).

Association between B vitamins, 1C metabolites, and cardiometabolic health

Higher betaine and glycine but lower choline and homocysteine concentrations were associated with a lower BMI and a favorable lipid panel (higher HDL-cholesterol, lower total/HDL-cholesterol, and lower triglycerides concentrations) in age- and sex-adjusted models (Figures 2 and 3, Supplemental Table 2). Diastolic blood pressure was positively associated with choline but inversely with glycine and serine concentrations in age- and sex-adjusted models. These associations largely remained significant with further adjustment for physical activity, supplementation (vitamin B or multivitamin), energy intake, and polypharmacy ($P < 0.05$) (Supplemental Table 3).

Serum folate demonstrated the most consistent association between vitamin B intake and status with cardiometabolic markers. Serum folate was inversely associated with BMI, fasting blood glucose, total/HDL-cholesterol, and triglycerides but positively with HDL-cholesterol concentrations (age- and sex-adjusted models, $P < 0.05$). Associations with BMI, HDL-cholesterol, and the total/HDL-cholesterol concentrations ratio remained significant when further adjusted for physical activity, supplementation (vitamin B or multivitamin), energy intake, and polypharmacy (Figure 2, Supplemental Table 2).

Limited associations between vitamins B or 1C metabolites and cognitive function

According to scores from COMPASS testing, higher glycine concentrations were associated with better global cognition (β=1.340, P =0.017), episodic memory (β=1.396, P =0.016), and spatial memory (β=1.394, P =0.027) z-scores in fully adjusted models (Supplemental Table 3).

TABLE 4
Effect of interaction between one-carbon metabolites and apolipoprotein ε4 genotype on cognitive performance in multivariate linear regression models

Metabolite (plasma)	Model covariates	Global cognition		Attention		Episodic memory		Executive function		Spatial memory		Working memory	
		• β	• *P*		• *P*	• β	• *P*	• β	*P*	β	*P*	β	*P*
Betaine	• Betaine	• 0.002	• 0.800	• -0.003	• 0.714	• <0.001	• 0.983	• 0.002	• 0.794	• 0.008	• 0.292	• 0.006	• 0.455
	• ApoE ε4	• -0.509	• 0.211	• -0.455	• 0.304	• 0.515	• 0.219	• -0.049	• 0.913	• -0.477	• 0.295	• 0.129	• 0.772
	• Interaction	• 0.011	• 0.343	• 0.013	• 0.293	• 0.007	• 0.524	• 0.005	• 0.699	• 0.008	• 0.542	• -0.003	• 0.918
• Choline	• Choline	• 0.001	• 0.976	• -0.026	• 0.437	• 0.030	• 0.334	• 0.009	• 0.797	• -0.021	• 0.554	• -0.016	• 0.622
	• ApoE ε4	• -0.335	• 0.457	• -0.333	• 0.498	• 0.006	• 0.989	• 0.125	• 0.799	• -0.817	• 0.107	• -0.485	• 0.326
	• Interaction	• 0.024	• 0.646	• 0.038	• 0.499	• -0.030	• 0.564	• -0.001	• 0.991	• 0.072	• 0.213	• 0.067	• 0.231
• Cysteine	• Cysteine	• -0.002	• 0.705	• -0.007	• 0.144	• 0.004	• 0.362	• 0.001	• 0.896	• -0.007	• 0.161	• <0.001	• 0.946
	• ApoE ε4	• -0.367	• 0.636	• -0.569	• 0.499	• 0.498	• 0.531	• 0.237	• 0.778	• -2.038	• 0.019*	• -0.488	• 0.565
	• Interaction	• 0.002	• 0.764	• 0.006	• 0.501	• -0.008	• 0.337	• -0.001	• 0.886	• 0.019	• 0.033#	• 0.006	• 0.493
• Dimethylglycine	• Dimethylglycine	• -0.138	• 0.717	• -0.319	• 0.444	• -0.146	• 0.722	• 0.029	• 0.489	• -0.491	• 0.251	• 0.506	• 0.227
	• ApoE ε4	• -0.613	• 0.017*	• -0.305	• 0.279	• -0.587	• 0.027	• -0.192	• 0.493	• -0.823	• 0.004*	• -0.064	• 0.820
	• Interaction	• 1.640	• 0.037#	• 1.004	• 0.243	• 1.129	• 0.163	• 1.095	• 0.201	• 2.101	• 0.017#	• 0.563	• 0.513
• Glycine[1]	• Glycine	• 1.619	• 0.011*	• 0.988	• 0.155	• 1.689	• 0.010*	• 0.633	• 0.326	• 2.071	• 0.004*	• -0.610	• 0.382
	• ApoE ε4	• 1.783	• 0.441	• -0.071	• 0.978	• 1.969	• 0.407	• 1.451	• 0.569	• 5.052	• 0.051	• -0.203	• 0.426
	• Interaction	• -0.827	• 0.412	• 0.034	• 0.098	• -0.961	• 0.353	• -0.577	• 0.602	• -2.280	• 0.043#	• 0.921	• 0.408
• Homocysteine	• Homocysteine	• -0.266	• 0.584	• -0.338	• 0.516	• 0.355	• 0.492	• -0.317	• 0.531	• -1.226	• 0.024*	• -0.260	• 0.631
	• ApoE ε4	• -0.099	• 0.745	• 0.292	• 0.371	• -0.236	• 0.466	• 0.151	• 0.633	• -0.914	• 0.007*	• 0.274	• 0.418
	• Interaction	• 0.104	• 0.902	• -0.714	• 0.427	• -0.035	• 0.969	• 0.022	• 0.980	• 2.395	• 0.011#	• -0.227	• 0.807
• Methionine	• Methionine	• <0.001	• 0.994	• -0.017	• 0.382	• 0.009	• 0.638	• 0.024	• 0.234	• 0.004	• 0.847	• -0.016	• 0.436
	• ApoE ε4	• -0.074	• 0.920	• -0.717	• 0.374	• -0.096	• 0.900	• 0.919	• 0.254	• -0.152	• 0.855	• 0.582	• 0.472
	• Interaction	• -0.003	• 0.932	• 0.035	• 0.373	• -0.008	• 0.828	• -0.038	• 0.313	• -0.003	• 0.948	• -0.023	• 0.539
• SAM	• SAM	• -0.002	• 0.093	• -0.003	• 0.028*	• -0.001	• 0.390	• 0.001	• 0.600	• -0.002	• 0.176	• -0.001	• 0.465
	• ApoE ε4	• -0.685	• 0.007#	• -0.059	• 0.032*	• -0.654	• 0.012*	• 0.048	• 0.862	• -0.579	• 0.042*	• -0.159	• 0.566
	• Interaction	• 0.004	• 0.016#	• 0.004	• 0.019#	• 0.003	• 0.090	• 0.001	• 0.772	• 0.003	• 0.145	• 0.002	• 0.323
• Serine	• Serine	• -0.001	• 0.739	• -0.002	• 0.741	• 0<0.001	• 0.934	• <0.001	• 0.948	• 0.001	• 0.857	• -0.007	• 0.151
	• ApoE ε4	• -0.681	• 0.285	• -0.300	• 0.665	• -0.770	• 0.239	• 0.406	• 0.558	• -0.581	• 0.416	• -0.733	• 0.291
	• Interaction	• 0.007	• 0.385	• 0.004	• 0.668	• 0.006	• 0.427	• -0.004	• 0.673	• 0.005	• 0.595	• 0.010	• 0.229

β estimate and *P* values presented for main effects (one-carbon metabolite concentration and ε4 allele carrier) and the interaction between the main effects on cognitive outcomes. All models are adjusted for age, sex, education, batch effects, energy intake, physical activity, history of anxiety/depression, and supplement use.

* Indicates a significant main effect of metabolite concentrations or apolipoprotein E genotype

# Indicates a significant interaction effect ($P < 0.05$). Significant interactions were followed with post-hoc analyses, which were no longer significant with Tukey's adjustment for multiple comparisons.

[1] Fit as log-transformed variables in models. Abbreviations: SAM, *S*-adeonsylmethionine.

Higher vitamin B_6 intake was associated with an increased risk for mild cognitive impairment based on MoCA test scores in the fully adjusted generalized linear model (Poisson family, risk ratio=1.295, 95% CI=1.04 – 1.60, P =0.020), though not in a model adjusted for age, sex, and education only (P =0.099) (Table 3).

No other metabolites or B vitamins were associated with cognitive performance in COMPASS testing or with altered risk of mild cognitive impairment according to MoCA scores. Similar findings are reported in sensitivity analyses irrespective of vitamin B or multivitamin and mineral supplement use (Supplemental Tables 4 and 5). Interactions with *APOE*-ε4 status did not remain significant after Tukey's adjustment for multiple comparisons (Table 4).

Discussion

This is a cross-sectional, secondary analysis of the REACH cohort of older adults in New Zealand, including vitamins B, a comprehensive panel of 1C metabolites (including those involved in folate, methionine, choline oxidation, and transsulfuration pathways), cardiometabolic risk markers, and cognitive function. We identified clear associations between serum folate and 1C metabolites involved in the choline oxidation pathway (choline, betaine, homocysteine) with cardiometabolic markers but limited associations with cognitive function. Higher glycine concentrations were consistently associated with a favorable cardiometabolic risk profile and modestly associated with better global cognitive performance, episodic memory, and spatial memory in a cognitive battery (COMPASS system).

The majority (> 90%) of participants in the REACH study met dietary requirements for riboflavin, vitamins B_6 and B_{12}, though we did find a relatively high prevalence of dietary folate inadequacy, with 22% of males and 33% of females not meeting the estimated average requirement of 320μg/d. The New Zealand food supply chain was subject to voluntary fortification of folic acid (including breads and cereals) and other vitamins B at the time of this study, and dietary analyses include vitamin B intake from both natural and fortified food sources but do not reflect contributions from regular or occasional supplement use. To provide some context to this cohort, average nutrient intakes are comparable with other cohorts of older adults examining the relationship between vitamins B and cognition in the UK [59] and the United States [60], though they do contrast with findings from the latest Adult Nutrition Survey in New Zealand (2008-2009) which shows a significantly greater proportion of the population aged >70 y with inadequate intakes of riboflavin (males: 15.4%, females: 18.7%), vitamin B_{12} (males: 3.8%, females: 27.0%), and vitamin B_6 (males: 28.8%, females, 53.0%) [61]. The REACH population is, therefore, a healthy group of early aging adults (65 – 74 y, 65% female) with largely adequate vitamin B intakes compared with the general aging population both in New Zealand and globally [62].

There is a wealth of evidence supporting the association between folate, vitamin B_{12}, and homocysteine with cognitive performance in older adults (6,7,16,17,8–15), and it is generally agreed that associations are stronger in the context of dietary or biochemical inadequacy ranges [63–65]. It is not entirely surprising then, that associations between folate, vitamin B_{12}, and homocysteine with cognition were not replicated in the current study. Lower dietary intakes or status within generally adequate ranges may, however, be more relevant in tracking cognitive trajectories. For example, lower dietary intakes of vitamin B_6 were associated with a greater risk of accelerated cognitive decline over a 4-y period in a cohort of older adults with similar average dietary intakes to the REACH cohort at baseline (59).

Glycine was modestly associated with better global cognitive performance, episodic memory, and spatial memory z-scores in the COMPASS cognitive battery. Interpretation of this finding is somewhat limited by difficulties in disentangling the role of glycine within 1C metabolism at this point in time. Glycine is the end-product following the degradation of metabolites involved in betaine-dependent homocysteine remethylation and is interconverted with serine in the cytosol and mitochondria. Glycine is also a substrate for glycine N-methyltransferase, thus implicated in maintaining the balance of *S*-adenosylmethionine and *S*-adenosylhomocysteine, or methyl group availability [28]. Indeed, *S*-adenosylmethionine-dependent methyl group transfers are critical for maintaining cognitive function, involving the methylation of proteins, membrane phospholipids, DNA, and neurotransmitters, and impaired methylation capacity is thought to drive the relationship between B vitamin inadequacy or elevated homocysteine with cognitive decline [66]. However, whether this mechanism extends to the involvement of glycine remains unclear without analysis of methylation capacity. Plausibly, the association between glycine and cognitive function could also stem from involvement in pathways outside of 1C metabolism. For instance, antioxidant imbalances, which are considered a feature of Alzheimer's disease onset and progression [67,68], and glycine has antioxidant capabilities through the production of glutathione [69]. The connection between glycine and health in older adults is likely complex and warrants future investigations to disentangle underlying mechanisms.

Other metabolites of the choline oxidation pathway were not associated with cognitive parameters despite previous research demonstrating associations between higher dietary choline intake [21,22] and status [23] or DMG concentrations [24] with improved cognitive performance and protection against cognitive decline. Similarly, the interconnected regulation of folate and choline metabolism is thought to be integral in the trajectory of cognitive aging according to pre-clinical models [70–72] but was not supported by findings in this cohort. The lack of associations may again be related to the largely adequate nature of vitamin B status of the REACH cohort, with > 90% of the population meeting requirements for riboflavin, vitamins B_6 and B_{12} intake. The impact of choline or betaine on homocysteine concentrations appears to be more pronounced with lower B vitamin status [73–75], suggesting that homocysteine remethylation is more dependent on betaine as a methyl donor when B vitamin intake is restricted. This finding could theoretically be extended to associations with clinical outcomes like cognitive performance, whereby associations between choline metabolites and cognition may be more pronounced in the context of vitamin B inadequacy.

We did not find any interactive effects of ApoE genotype on the relationship between vitamins B or 1C metabolites with cognitive performance in this population, where 26% were *APOE*-ε4 allele carriers, which does contrast with previous reports showing stronger associations between higher

homocysteine concentrations [39,40] or lower vitamin B_{12} status [10,37,38] in *APOE*-ε4 allele carriers [39]. This was an exploratory and likely inadequately powered analysis in the REACH population compared with other cohorts with samples of at least 500 participants where differences according to ApoE genotype have been detected in populations with a similar frequency of *APOE*-ε4 allele carriers (ranging from 16% to 32%) [10,37,38].

Higher vitamin B_6 intake was associated with a greater risk of having mild cognitive impairment in a fully adjusted model. Although the evidence base is not as robust compared with that for folate or vitamin B_{12}, the literature generally supports an association between higher vitamin B_6 intake or status with better cognitive performance [76], reduced risk for cognitive decline [59], greater gray matter volume [65], and preservation of cortical structures in the brain [77], although others have found no associations [6]. A similar trend was seen in sensitivity analyses conducted for those participants not taking vitamin B or multivitamin supplements ($P = 0.075$ in the fully adjusted model). Given that this relationship was only significant in fully adjusted models and biochemical measures of vitamin B_6 status were not obtained to evaluate functional status, this creates difficulties in drawing firm conclusions. Regardless, this finding does raise the point of complexities associated with narrowing broader dietary patterns down to a focus on individual nutrients. For instance, higher vitamin B intake may represent the 'Western' style dietary pattern from the primary outcome analysis of the REACH study [42], characterized by processed meats or meat pies (a source of vitamin B_{12}), sweetened cereals (often fortified with B vitamins), cheese (a source of riboflavin), among other ultra-processed foods. Although not seen in the REACH study, such Western-style dietary patterns are often associated with poorer cognitive outcomes [78,79]. Future analyses which aim to understand the relative contribution of processed foods to vitamin B intake and status and to disentangle the positive and detrimental impacts this might have on brain health are warranted.

Associations between 1C metabolites and cardiometabolic markers were more extensive than the very few associations found with cognitive outcomes. For example, higher betaine and glycine concentrations were associated with a favorable cardiometabolic risk profile, whereas the inverse was true for cysteine, choline, and homocysteine, and this association was most consistent for glycine and choline across different parameters (i.e., BMI, fasting blood glucose, cholesterol, triglycerides concentrations, and blood pressure). These findings are similar to those previously reported for glycine [56,80–82], betaine and choline [26,27,56,83–85], cysteine [56,86,87], and homocysteine [88,89]. Associations between metabolites and cardiometabolic health might indicate earlier perturbations in the broader regulation of 1C metabolism or a more sensitive indicator of an 'optimal' profile of 1C metabolites. This observation has relevance for monitoring health status and directing intervention strategies in older adults. Future research is needed to understand how 1C metabolites might contribute to the progression of metabolic disturbances to cognitive impairment, allowing for appropriate stratification of nutritional, metabolic, and cognitive status.

The current study has a number of strengths and limitations important to the interpretation of findings. The strength of this analysis lies in a broader profile of vitamins B and 1C metabolites than typically measured, using an analytical technique that overcomes the challenges of quantifying heterogeneous molecular structures and concentration ranges within an integrated metabolite profile [49]. It is important to note that the technique used here does result in lower homocysteine concentrations than expected ranges (between 5 – 15μM is considered normal [90]). This can be explained by lower concentrations of Tris (2-carboxyethyl) phosphine used in sample preparation compared with other published methods [91], an agent used to reduce disulfide bonds present in cystine and homocysteine to be quantified as cysteine and homocysteine, respectively. Dietary assessment was thorough with respect to the detailed direction provided to complete the 4-d food records, an experienced research team, and the rigorous internal checks performed. Food records are preferred over other assessment methodologies for quantifying absolute nutrient intake [92] and allow for estimates of vitamin B intake from both natural and fortified food sources in New Zealand in the current study. Food records are, however, subject to social and reactivity bias and do not necessarily reflect habitual diet over longer periods of time [92], though our previous reports do show good agreement between vitamin B intake reported from both food records and a food frequency questionnaire estimating intake over the past 3 mo in the REACH cohort [93]. Although the research team did probe for details on fortified foods/beverages, we acknowledge that a purpose-designed food frequency questionnaire for vitamin B intake, such as that used by other research groups [59] and adapted to the New Zealand food supply, would have further strengthened assessment of nutrient intake from fortified foods/beverages.

Convenience sampling was used for the current study, and extrapolations must be drawn carefully given the unbalanced sex distribution with only 35% males. Differences in 1C metabolite profiles according to sex are important to note here, with males showing higher average concentrations for most 1C metabolites except for glycine and serine, which were higher in females. These sex-related differences correspond with previous findings from our own lab group in older adults [94] and other groups across broad age ranges [95,96]. Future efforts to tease apart any differential relationships between 1C metabolites and cognitive or cardiometabolic health according to sex are warranted in populations better balanced for sex than the current cohort. Similarly, findings from this study should also be confirmed in populations with greater sociodemographic diversity, and potential differences in populations with a higher prevalence of nutritional inadequacy or metabolic dysregulation should be considered.

This research provides insight into the nuanced relationships between B vitamins, 1C metabolites, cardiometabolic health, and cognitive function in a population of healthy older adults. Metabolites relevant to the choline oxidation pathway were associated with cardiometabolic markers, with higher betaine and glycine but lower choline and homocysteine concentrations associated with lower BMI and a favorable lipid panel. Higher glycine concentrations were also associated with better global cognitive

performance, episodic memory, and spatial learning, though future research is needed to disentangle the role of glycine within 1C metabolism in this context. These findings reinforce the need to measure a broader profile of 1C nutrients when monitoring the nutritional and health status of aging populations and should be confirmed in more diverse aging cohorts with respect to socio-demographic, nutritional, and health status.

Author contributions

The authors' responsibilities were as follows—KB, CC, PvH, BJ, WS, A-LMH, JC, CH-R, and DC-S designed research; KB, CC, PvH, KM, and NG conducted the research; NG analyzed the data; N.G., wrote the original draft; KB, CC, PvH, BJ, WS, JC, CH-R, KM, AMM, NCR, and DC-S edited and reviewed the paper. All authors have responsibility for final content. All authors have read and approved the final manuscript.

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Data availability

Data described in this manuscript will be made available upon request, pending reasonable application and approval.

Conflict of interest

The authors declare they have no conflict of interest.

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Appendix A. Supplementary data

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