**Title: Association of choline and B vitamins with incident of dementia: a large population-based prospective cohort study**

**Word count: 3869; Figure: 1; Tables: 4.**

**Abstract**

**Objective:** Previous analysis found that choline and B vitamins are associated with the risk of dementia, however, limited studies has investigated the effects of dietary choline or B vitamins intake on cognition function and the risks of incident dementia, and the results are inconsistent. We aimed to explore the potential associations of choline and B vitamins (including folate, vitamin B6 and B12) with the incidence of dementia among the people in the United Kingdom used data from the UK Biobank.

**Methods:** This prospective study enrolled 126 414 participants after screened. Dietary choline and B vitamins intakes were assessed using quantitative 24-hour dietary recall questionnaire. Diagnoses of dementia was identified using the ICD-9 or ICD-10 coding system. Cox proportional hazards regression, logistic regression and restricted cubic splines were used to analyze the association between choline and B vitamins intake and dementia or cognitive function.

**Results:**Among 126 414 participants, 55.8% were female and the mean age at baseline was 56.1 (SD 7.8) years old. Over a median of 10.6 years follow-up, 930 cases of incident dementia (including 385 AD) were recorded. We found nonlinear associations of choline and B vitamins intake with incident dementia and AD. Compared with the lowest intake quartile, participants in the 2nd to 4th quartiles had a 24% to 38% lower risk of incident dementia after adjustment for potential confounders. We found that among individuals with poor performance, moderate intake of choline and B vitamins were associated with lower risk of dementia with HRs of 0.68 (95% CI, 0.51, 0.92) for total choline in the 2nd quartile, 0.65 (95% CI, 0.48, 0.87) and 0.60 (95% CI, 0.43, 0.83) for folate in the 2nd and 3rd quartile, 0.60 (95% CI, 0.43, 0.83) for vitamin B6 in the 3rd quartiles and 0.66 (95% CI, 0.45, 0.97) for vitamin B12 in the 4th quartile. The association of choline and B vitamins with dementia among individuals with better performance was less consistent and conclusive, with wide 95% CIs that included 1.0 in most cases.

**Conclusions:**In this cohort study, moderate consumption of choline and B vitamins (including folate, vitamin B6 and vitamin B12) could reduce the risk of dementia, especially among those with poor cognitive performance.

**Key Words:** choline; folate; vitamin B6; vitamin B12; dementia; AD; cognitive performance

**Introduction**

According to statistics up to 2015, 46.8 million people worldwide were living with dementia (1), with the number expected to reach an estimated 152.8 million by 2050 (2). Dementia, including Alzheimer's disease (AD), Vascular dementia (VaD), and other progressive neurocognitive disorders, leads to a gradual decline in cognitive and functional abilities until death (3). The prevalence of dementia imposes a remarkable burden on society, patients and their families, making it one of the most pressing global public health and social care challenges.

Nutrition, especially through essential nutrients like choline, folate, vitamin B6, and vitamin B12, plays a vital role in preventing dementia (4-6). Choline and folate serve as methyl donors for homocysteine (Hcy), a well-established risk factor for dementia (4, 7). Intake of choline and folate in the diet or through supplements provides carbon groups to Hcy, allowing its degradation to cysteine or its methylation to form methionine. Methionine is a precursor for phospholipid synthesis, critical for the integrity of cell membranes and intracellular signaling (8, 9). In this process, vitamin B6 and B12 act as essential coenzymes (8-11). Additionally, choline acts as a precursor for acetylcholine, a neurotransmitter associated with memory, in the cholinergic neural networks (8). Animal experiments indicated the protective effects of dietary choline and B vitamins supplementation on reducing the brain β amyloid load and microglia activation, enhancing neuroplasticity, activation of anti-oxidative mechanisms and improving cognitive performance (12-14).

However, there are limited studies that investigated the effects of the amount of dietary choline or B vitamins intake on cognition function and the risks of incident dementia, and the findings are inconsistent (4, 5, 15-17). Certain prior analyses have indicated that the supplementation of choline and B vitamins led to significant enhancements in specific cognitive domains and exhibited an inverse correlation with dementia (16-19). However, conflicting reports suggest no such associations (15, 20, 21). Additionally, the majority of these studies relied on small sample sizes, potentially compromising the accuracy and representativeness of their findings.

To fill the significant gaps in existing knowledge, we implemented our investigation using data from the UK Biobank to explore the potential associations of choline and B vitamins (including folate, vitamin B6 and B12) with the incidence of dementia. Our hypothesis proposed that intake of choline and B vitamins could offer variable effects on the incidence of dementia within this population.

**Methods**

**Study design and participants**

This study employed a prospective, population-based cohort design using data from the UK Biobank. The cohort consisted of a total of 502 413 participants, aged 38-73, who were recruited through postal invitations from 2006 to 2010. These participants attended one of 22 study centers located in England, Scotland, or Wales. Comprehensive data collection methods were utilized, including completion of a touchscreen questionnaire, a nurse-led interview, physical measurements, and provision of biological samples. Detailed information regarding these procedures can be found elsewhere (22, 23). All participants provided written informed consent for the collection, analysis, and linkage of their data with hospital admissions, cancer registries, and death registries.

For this analysis, participants who had completed the online 24–hour dietary recall questionnaire on at least two occasions were eligible for inclusion. After excluded people without twice 24-hour dietary recalls (n=374 621), lost to follow-up (n=1 298), and with dementia at baseline (n=80), a total of 126 414 participants were eligible for inclusion in the study (Figure S1).

**Assessment of dietary choline and B vitamins**

A web-based 24-hour dietary instrument (Oxford WebQ) was used to collect detailed dietary data. The Oxford WebQ was validated against an interviewer-administered 24-hour recall questionnaire (24) and collected information on the quantities of up to 206 widely consumed food items and 32 types of drinks consumed over the previous day (25). Participants with valid e-mail addresses were invited to complete a dietary questionnaire at baseline and were followed up to four times over 1 year to account for seasonal variations in dietary intake between April 2009 and June 2012. Participants with at least two assessments were retained for analysis to better reflect usual intakes (26). We estimated nutrient intake using McCance and Widdowson’s Composition of Foods (27). Because no information on choline and betaine values in UK foods exists, the values for these nutrients in the diet are based on the USDA database (28, 29). Total choline is formed from free choline, phosphocholine (PCho), phosphatidylcholine (PtdCho), glycerophosphocholine (GpCho), and sphingomyelin (Sphingo). B vitamins included folate, vitamin B6, and vitamin B12, supplements were excluded as part of the definition of B vitamins intake. Considered the changes of intakes during follow-up, dietary choline and B vitamins were calculated from the average of all surveys.

**Assessment of dementia and cognitive function**

The primary outcomes of interest in this study were incident dementia. The outcomes of the study were determined by examining hospital admission records and death certificate records obtained from the Hospital Episode Statistics for England, the Scottish Morbidity Record data for Scotland, and the Patient Episode Database for Wales. Diagnoses were identified using the ICD-9 or ICD-10 coding system. We defined outcomes according to the ICD-9 and ICD-10: dementia (290.2, 290.3, 290.4, 291.2, 294.1, 331.0, 331.2, 331.5, A81.0, F00, F01, F02, F03, F05.1, G30, G31.1, G31.8) and AD (331.0, F00, G30) (Table S1). The follow-up period for the study extended from March 21, 2006, to October 31, 2021. Person-years for each participant were calculated from the date of baseline assessment until the occurrence of dementia, date of death, or the end of the follow-up period (September 30, 2021, for England and Wales, and October 30, 2021, for Scotland), whichever came first. Incident rates were calculated as the number of events per 1000 person-years.

The secondary outcomes of interest in this study were cognitive function at baseline. Cognitive function was assessed by cognitive tests were included in UK Biobank, all of which were administered via computerized touchscreen interface, including trail making test, verbal-numerical reasoning and prospective memory (30). The trail making test measured psychomotor speed and aspects of executive function, which was not language dependent (31, 32). The test had been validated and recommend by The International Cognition and Cancer Task Force (ICCTF) as one of methods for evaluating cognitive function (33, 34). The test of verbal-numerical reasoning was a test of fluid intelligence, which was used to asses verbal and numerical problem-solving and had a test–retest reliability (35, 36). Prospective memory was measured by symbol digit match, which was a part of the Wechsler Adult Intelligence Scale (third edition), and validation studies had shown that the score correlated with measures of future cognitive decline (37-39). Detailed information and criterion of the cognitive tests is shown in Table S2. The sample sizes for the different tasks vary. Then we divided the participants into better or poor performance based on three cognitive tests. "Poor performance" was defined as any of three-test performed poorly and "Better performance" was defined as all three-test performed well.

**Assessment of covariates**

We used baseline questionnaires to assess the following potential confounders: age, sex, ethnicity, Townsend deprivation index (TDI), education level, centers, body mass index (BMI), waist circumference, healthy dietary pattern, physical activity, alcohol consumption, smoke status, pack-years of smoking, sleep time, family history of dementia, hypertension, diabetes, stroke, depression, Parkinson, use of cholesterol-lowering drugs, aspirin drugs, diabetes drugs, blood pressure drugs, vitamin and mineral supplements, dietary intake of vitamin D and total energy. The TDI served as a measure of material deprivation within the population, with negative values indicating less deprived areas. Hypertension was defined as a self-reported history of hypertension, systolic blood pressure of 140 mm Hg or higher, diastolic blood pressure of 90 mm Hg or higher, or use of antihypertensive drugs. According to the guidelines, we categorized participants into low, moderate, and high activity level based on categorical criteria (40). Consistent with previous UK Biobank studies (41, 42), a healthy dietary pattern was defined as the adequate consumption of at least four of seven specified food groups. The general definition of a pack-year of smoking was the number of cigarettes smoked per day divided by 20 and multiplied by the number of years of smoking. Medication use for cholesterol, aspirin, diabetes, hypertension and vitamin and mineral supplements was collected by self-reported touch screen questionnaire or verbal interviews. Further details regarding the assessment of these factors can be found on the UK Biobank website (<http://www.ukbiobank.ac.uk>).

**Statistical analysis**

Detailed information on the missing covariates is presented in Table S3. To account for the missing values, multiple imputation by chained equations (MICE) method was used, and 10 datasets were created through this imputation process. All variables, including the outcomes, were included in the multiple imputation model, ensuring a comprehensive imputation of missing values. Baseline characteristics are presented as the mean (standard deviation [SD]) for continuous variables and number (percentage [%]) for categorical variables.

Participants were divided into quartiles according to intake of choline and B vitamins, respectively. Cox proportional hazards regression was employed to estimate the hazard ratios (HRs) and corresponding 95% confidence intervals (95% CIs) for incident dementia and AD in relation to total choline, folate, vitamin B6, vitamin B12. Similar calculations were conducted to assess the associations of choline-contributing compounds and betaine with incident dementia. Furthermore, dose-response relationships analyzed through nonparametrically restricted cubic spline regression, with knots positioned at the 25th, 50th, and 75th percentiles, exploring the association of choline and B vitamins with incident dementia and AD. Subsequently, logistic regression was employed to estimate the odds ratios (ORs) and 95% CIs regarding the association of choline and B vitamins with cognitive function.

In the analysis, several covariates were adjusted for. In model 1, adjustments were made for baseline age (years, continuous), sex (male or female), and energy (kcal/d, continuous) intake; model 2 included further adjustments for TDI (continuous), ethnicity (white, or others), education level (degree, or not degree), centers (Scotland, Wales, or England), BMI (kg/m2, continuous), waist circumference (cm, continuous), healthy dietary pattern (yes or no), physical activity level (low, moderate, or high), alcohol consumption (g/d, continuous), smoking status (current, former, or never), pack-years of smoking (years, continuous), sleep time (hours/d, continuous), family history of dementia (yes or no),diseases of baseline (yes or no; hypertension, diabetes, stroke, depression, Parkinson), drug use (yes or no; cholesterol lowering, aspirin, diabetes, blood pressure), use of vitamin and mineral supplement (yes or no; vitamin A, vitamin B, vitamin C, vitamin D, vitamin E, folic acid, or multivitamins/minerals), percentage of energy (%, continuous) from fat, protein, carbohydrate, sugar, vitamin D (μg/d, continuous). Choline, folate, vitamin B6 and vitamin B12 were mutually adjusted.

To evaluate the robustness of our findings, we conducted several sensitivity analyses. We excluded participants who use of B vitamins supplements, experienced an outcome event during the first three years of follow-up, had depression and stroke at baseline or had missing covariates. Furthermore, we assessed the competing risk of non-dementia-related death on the association between choline, B vitamins and the risks of dementia. Additionally, we redefined the better or poor performance based on three cognitive tests. "Poor performance" was defined as two or more of three-test performed poorly and "better performance" was defined as zero or one of three-test performed poorly.

Analyses were performed using R statistical software (version 4.3.1 for Windows). Statistical tests were two-sided, and *P* values less than 0.05 were considered statistically significant.

**Results**

**Baseline characteristics**

Table 1 shows the demographic characteristics of the 126 414 participants. At baseline, the mean age was 56.1 (SD 7.8) years, with 70 486 (55.8%) being female. The mean (SD) intake of total choline, betaine, folate, vitamin B6 and vitamin B12 were 304.4 (SD 119.1) mg/d, 146.7 (SD 96.7) mg/d, 303.2 (SD 98.2) μg/d, 2.2 (SD 0.6) mg/d, and 6.6 (SD 3.8) μg/d respectively. The intake of betaine and choline-contributing compounds including free choline, phosphatidylcholine, sphingomyelin, phosphocholine and glycerophosphocholine were 146.7 (96.7) mg/d, 96.3 (SD 45.9) mg/d, 146.0 (SD 71.4) mg/d, 9.4 (SD 4.9) mg/d, 10.7 (SD 4.8) mg/d, and 41.2 (SD 16.0) mg/d respectively. Overall, 47.8% of the participants were highly educated, 11.4% had a family history of dementia, and 37.0% of the participants took vitamins and mineral supplements. Over a median of 10.6 years follow-up, 930 cases of incident dementia (including 385 AD) were recorded.

**Intake of choline,** **choline-related compounds and B vitamins with incident dementia and AD**

Cox models with penalized splines showed statistically significant nonlinear associations for total choline, folate, vitamin B6 and vitamin B12 with dementia (*p*-nonlinear< 0.001, Figure 1). The lowest probabilities for incident dementia were of 350.2 mg/d, 329.9 μg/d, 2.3 mg/d and 9.1μg/d for choline, folate, vitamin B6 and vitamin B12 intake, respectively. Compared with those in the lowest intake quartile, participants in the 2nd to 4th intake quartiles had a lower risk of incident dementia, with HRs of 0.76 (95% CI, 0.62 to 0.92) for choline in the 2nd quartile, 0.73 (95% CI, 0.59 to 0.90) and 0.72 (95% CI, 0.58 to 0.90) for folate and vitamin B6 in the 3th quartile, and 0.62 (95% CI, 0.49 to 0.80) for vitamin B12 in the 4th quartile (Table 2).

We also found nonlinear associations between choline, B vitamins and AD. The lowest probabilities for incident AD were of 338.6 mg/d, 329.9 μg/d, and 8.5μg/d for choline, folate, and vitamin B12 intake, respectively. Compared with those in the lowest intake quartile, participants in the 2nd to 4th intake quartiles also had a lower risk of incident AD, with HRs of 0.72 (95% CI, 0.53 to 0.98) and 0.73 (95% CI, 0.53 to 0.99) for choline and folate in the 2nd quartile, and 0.54 (95% CI, 0.37 to 0.80) for vitamin B12 in the 4th quartile. However, the association of vitamin B6 with AD was less conclusive (Figure S2 and Table S4).

We then examined the associations between choline-related compounds and dementia. Our analysis revealed that 2nd to 4th quartiles consumption of free choline, PtdCho, Sphingo and betaine was linked to a 7% to 23% decreased risk of dementia compared to the first quartile. When we combined intake of total choline and betaine, participants in the 3rd intake quartile had an obviously lower risk of incident dementia, with HR of 0.78 (95% CI, 0.64 to 0.96) (Table S5).

**Intake of choline and B vitamins and** **cognitive performance**

In the subset, 65 179, 76 098 and 73 533 participants respectively completed the trail making test, verbal-numerical reasoning test and prospective memory test. We found that moderate intake of choline and vitamin B12 were associated with better performance on all three cognitive tests. Compared to the lowest intake quartile for choline and vitamin B12, intake in the 2nd to 4th quartiles was associated with a 6% to 18% reduced risk of poor cognitive performance. The association of folate and vitamin B6 with all three cognitive performance was less consistent and conclusive, with wide 95% CIs that included 1.0 in some cases. However, vitamin B6 was associated with a 5% lower risk of prospective memory in the 2nd quartile, and folate was associated with 8% to 12% lower of verbal-numerical reasoning in the 2nd to 4th quartiles (Table 3).

**Intake of choline and B vitamins and incident dementia** **across different cognitive performance at baseline**

In the subset, 13 756 and 63 301 participants were respectively considered as “better” and “poor” performance. We found that among individuals with poor performance, moderate intake of choline and B vitamins were associated with lower risk of dementia with HRs of 0.68 (95% CI, 0.51, 0.92) for total choline in the 2nd quartile, 0.65 (95% CI, 0.48, 0.87) and 0.60 (95% CI, 0.43, 0.83) for folate in the 2nd and 3rd quartile, 0.60 (95% CI, 0.43, 0.83) for vitamin B6 in the 3rd quartiles and 0.66 (95% CI, 0.45, 0.97) for vitamin B12 in the 4th quartile. The association of choline and B vitamins with dementia among individuals with better performance was less consistent and conclusive, with wide 95% CIs that included 1.0 in most cases. When we redefined the poor and better performance, the results remained largely unchanged (Table S6).

**Sensitivity analyses**

The results of the sensitivity analyses showed only minor variations from the primary findings after adjusting for multiple variables. Inverse associations were strengthened when we excluded participants using B vitamins supplements for folate and vitamins B12 (Table S7), and strengthened after excluded individuals with depression and stroke at baseline for vitamins B6 and vitamins B12 (Table S8). Excluding participants with incident dementia during the first three years of follow-up or those with missing values attenuated the associations for choline, folate, and vitamins B6 (Table S9, Table S10). The association between each B vitamin and choline intake and dementia remained largely unchanged when utilizing a competing risk regression model (Table S11).

**Discussion**

In this large, prospective and population-based cohort from the UK Biobank, we found nonlinear associations of choline, choline-related compounds (free choline, PtdCho, Sphingo and betaine), folate, vitamin B6 and vitamin B12 intake with incident dementia, and of choline, folate and vitamin B12 intake with incident AD. Moderate intake of choline and Bvitamins were associated with better cognitive performance at baseline, and inverse associated with incident dementia among those with poor cognitive performance at baseline. The results implied that moderate consumption of choline and B vitamins could reduce the risk of dementia, especially among those with poor cognitive performance at baseline.

Several studies have investigated the relationship between dietary choline intake and incident dementia, but with inconsistent findings (15, 17, 43). Our findings showed that participants who had a total choline intake ranging from 221 to 284 mg/d exhibited a lower risk of incident dementia and AD, which was in accord with a prior research study in Framingham Heart Study (FHS) (15). A placebo-controlled double-blind study also showed that choline supplementation changed human cholinergic functions in the nervous system and improved cognitive function (43). Nevertheless, in other similar research study conducted by Ylilauri et al. among Finnish people, no significant association was found between total choline and incident dementia or AD (17). Interestedly, the study revealed an inverse correlation between PtdCho and incident dementia. In our results, we also found that moderate PtdCho intake was associated with lower incident dementia significantly. We further noted that moderate intake of other choline-related compounds (including free choline, Sphingo and betaine) also had a lower risk of incident dementia. The discrepancy of results in these studies may be partly explained by the differences in methodology, like the study design, ethnic background, classification of choline intake. When we further analyzed populations with poor cognitive performance at baseline, we consistently observed that moderate intake of choline could substantially diminish the risk of dementia. To the best of our knowledge, we are the first to report these results within a population characterized by subpar cognitive performance, which underscores that choline not only serves as a preventive measure against dementia in the general population but is particularly beneficial for individuals with poor cognitive performance.

The current study revealed that the moderate folate intake was associated with lower risk of incident dementia or AD, aligning with the prevailing findings in most previous research (44-47). However, the results for vitamin B6 and B12 have been inconsistent. Our results are not entirely consistent with at least four study (5, 47-49). The null results from these studies are difficult to interpret because of limited sample size, brief follow-up duration, and insufficient representation of individuals with high vitamin intake. In a number of relatively large RCTs and observational studies that followed participants over several years, 3 studies highlighted positive effects of B vitamins intake on cognitive function (44, 49-51), which generally support our findings. As per the US guidelines, the recommended dietary allowance (RDA) for vitamin B6 ranges 1.3 to 1.7 mg/d, and for vitamin B12, it is 2.4 μg/d (52, 53). While, gastritis and other conditions may inhibit B vitamins absorption. In our study, the lowest probabilities for incident dementia were observed at 2.3 mg/d for vitamin B6 and 9.1μg/d for vitamin B12, respectively. Our results suggest that enhancing the recommended daily intake of vitamin B6 and vitamin B12 may be necessary to achieve optimal cognitive protection effects.

Our findings are biologically plausible. Choline serves as a precursor to membrane phospholipids (e.g., phosphatidylcholine), the neurotransmitter acetylcholine, and, the methyl group donor S-adenosylmethionine via betaine (13, 54). It helps prevent age-related memory decline and shields the brain from neuropathological changes linked to AD and neurological damage(13), an important reason is that choline can decrease microglia activation and regulate inflammation in the brain through a variety of way. Choline-induced effects are also associated with modifications in histone and DNA methylation in the brain, as well as alterations in the expression of genes encoding proteins crucial for learning and memory processing(55). A deficiency in B-vitamins leads to disruptions in one-carbon metabolism, resulting in elevated levels of homocysteine, reduced levels of S-adenosylmethionine (SAM), and increased levels of S-adenosylhomocysteine (SAH) (56-58). These conditions can trigger oxidative stress, leading to neurotoxicity and subsequent damage to neurons. It is well documented that folate in one-carbon metabolism plays a central role in the synthesis, repair, and methylation of DNA, where it acts as a methyl donor (57). Vitamin B12 serves as a co-factor for methionine synthase, and its deficiency can impact nucleotide synthesis, causing errors in DNA replication and promoting neuronal damage (57, 59). Similarly, a deficiency in B6 can affect the metabolism of homocysteine, inhibit the formation of downstream glutathione, and result in oxidative damage (60). In brief, dietary choline and its metabolite betaine, as well as B vitamins (such as folate and vitamin B6 and B12), by the regulation of homocysteine and methionine levels, play central roles in the prevention of the risk of dementia(55).

**Strengths and Limitations**

Our study has several strengths, such as its prospective design, a substantial sample size, multiple measurements of dietary information, and comprehensive analysis of dietary choline, choline-related compounds and B vitamins with incident dementia, AD, cognitive performance.

There are also several limitations. Firstly, due to lack of genetic data, we were unable to adjust for factors known to influence cognitive function, such as Apolipoprotein E ε4 (61). Nevertheless, Mendelian randomization studies on dementia are also prone to bias, and the results of genetic studies should be interpreted in light of the observational results. Secondly, the UK Biobank sample is representative of a high socioeconomic status; therefore, our results may vary in individuals with a lower socioeconomic status. Thirdly, our examination was limited to dietary choline and B vitamins, and we did not assess serum or red blood cell levels. Various factors, such as chronic inflammation or certain medications, may impact the absorption of these choline and vitamins. Consequently, the intake amounts may not accurately represent the quantity available for cellular processes. Nevertheless, we have considered the effects of numerous chronic diseases and medications. Fourthly, similar to other traditional observational cohort studies, it is challenging to completely eliminate the possibility of residual confounding arising from unmeasured or unknown factors. However, only an exceptionally influential unmeasured risk factor for mortality, combined with a substantial imbalance in prevalence among exposure groups, could account for such robust findings (62, 63).

**Conclusions**

In conclusion, we found that moderate consumption of choline and B vitamins (including folate, vitamin B6 and vitamin B12) could reduce the risk of dementia, especially among those with poor cognitive performance at baseline.

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# Figure 1. Multiple adjusted restricted cubic spline models for the associations of total choline (A), folate (B), vitamin B6 (C) and vitamin B12 (D) with incidence dementia

Multiple restricted cubic spline model is adjusted for age (years, continuous), sex (male or female) ethnicity (white, or others), Townsend deprivation index (continuous), education level (degree, or not degree), centers (Scotland, Wales, or England), BMI (kg/m2,continuous), waist circumference (cm, continuous), healthy dietary pattern (yes or no), physical activity level (low, moderate, or high), intake of alcohol (g/d, continuous), smoking status (current, former, or never), pack-years of smoking (years, continuous), sleep time (hours/d, continuous), family history of dementia (yes or no), diseases of baseline (yes or no; hypertension, diabetes, stroke, depression, Parkinson), drug use (yes or no; cholesterol lowering, aspirin, diabetes, blood pressure), use of vitamin and mineral supplement (yes or no; vitamin A, vitamin B, vitamin C, vitamin D, vitamin E, folic acid, or multivitamins/minerals), vitamin D (μg/d, continuous) and energy (kcal/d, continuous), percentage of energy (%, continuous) from fat, protein, carbohydrate, sugar. Choline, folate, vitamin B6 and vitamin B12 were mutually adjusted.