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ORIGINAL ARTICLE

Prospective study on dietary intakes of folate, betaine, and choline and cardiovascular disease risk in women

GW Dalmeijer[1,3], MR Olthof[1,2], P Verhoef[1,2], ML Bots[3] and YT van der Schouw[3]

[1]*Wageningen University, Wageningen, The Netherlands;* [2]*Wageningen Centre for Food Sciences, Wageningen, The Netherlands and* [3]*Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands*

Objective: To investigate the association between dietary intakes of folate, betaine and choline and the risk of cardiovascular disease (CVD).
Design: Prospective cohort study.
Subjects: A total of 16 165 women aged 49–70 years without prior CVD. Subjects were breast cancer screening participants in the PROSPECT–EPIC cohort, which is 1 of the 2 Dutch contributions to the European Prospective Investigation into Cancer and Nutrition (EPIC).
Methods: Each participant completed a validated food frequency questionnaire. Folate intake was calculated with the Dutch National Food Database. Betaine and choline intakes were calculated with the USDA database containing choline and betaine contents of common US foods. Data on coronary heart disease (CHD) events and cerebrovascular accident (CVA) events morbidity data were obtained from the Dutch Centre for Health Care Information.
Results: During a median follow-up period of 97 months, 717 women were diagnosed with CVD. After adjustment, neither folate, nor betaine, nor choline intakes were associated with CVD (hazard ratios for highest versus lowest quartile were 1.23 (95% confidence interval 0.75; 2.01), 0.90 (0.69; 1.17), 1.04 (0.71; 1.53), respectively). In a subsample of the population, high folate and choline intakes were statistically significantly associated with lower homocysteine levels. High betaine intake was associated with slightly lower high-density lipoprotein (HDL)-cholesterol concentrations.
Conclusion: Regular dietary intakes of folate, betaine and choline were not associated with CVD risk in post-menopausal Dutch women. However, the effect of doses of betaine and choline beyond regular dietary intake – for example, via supplementation or fortification – remains unknown.
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Keywords: betaine; choline; folate; homocysteine; cholesterol; cardiovascular disease

Introduction

A high plasma homocysteine concentration is associated with increased risk of cardiovascular disease (CVD) (Anonymous, 2002; Klerk *et al*., 2002), but causality of the association is not yet proven. Some clinical trials of homocysteine lowering through B-vitamin treatment support this hypothesis (Schnyder *et al*., 2001, 2002), but other more recent ones do not (Baker *et al*., 2002; Lange *et al*., 2004; Toole *et al*., 2004; Bonaa *et al*., 2006; HOPE 2 Investigators, 2006).

Homocysteine is a breakdown product of methionine, and can be further degraded to cysteine via vitamin B_6-dependent reactions. Alternatively, it can be remethylated into methionine, which requires a methyl group obtained from 5-methyltetrahydrofolate or from betaine (Figure 1) (Selhub, 1999. Supplementation with folic acid lowers plasma homocysteine by about 25% maximally (van Oort *et al*., 2003). Intervention studies in healthy volunteers suggest that also

Correspondence: Dr YT van der Schouw, University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, STR 6.131, PO Box 85500, 3508 GA Utrecht, The Netherlands.
E-mail: y.t.vanderschouw@umcutrecht.nl
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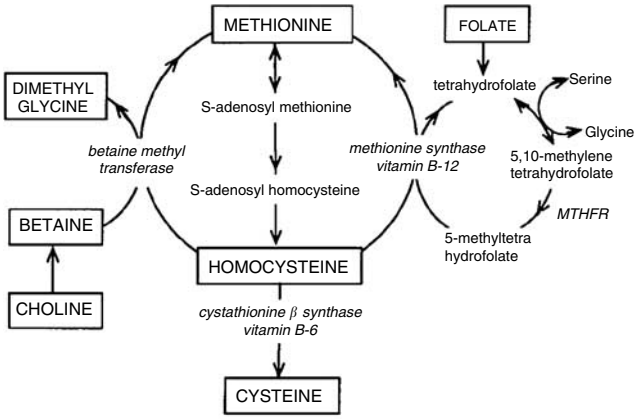

Figure 1 Schematic representation of homocysteine metabolism. MTHFR = 5,10-methylenetetrahydrofolate reductase.

supplementation with high doses of betaine (1.5–6 g/day), or its precursor choline (2.6 g/day, supplemented as phosphatidylcholine, the form in which choline occurs in foods) lowers fasting concentrations of plasma homocysteine (Schwab *et al*., 2002; Olthof *et al*., 2003, 2005a; Steenge *et al*., 2003).

Unlike folate, betaine and choline supplementation also lower plasma concentrations of homocysteine after a methionine load (Olthof *et al*., 2003, 2005a; Steenge *et al*., 2003). Both fasting homocysteine and the decrease of homocysteine concentrations after a methionine loading test predict the risk of CVD (Graham *et al*., 1997; Verhoef *et al*., 1999). Consequently, betaine and choline may have additional value for prevention of CVD relative to folic acid, which lowers only fasting homocysteine. On the other hand, studies in humans have shown that betaine supplementation above 1.5 g/day increases low-density lipoprotein (LDL)-cholesterol and triacylglycerol concentrations and phosphatidylcholine supplementation increases triacylglycerol concentrations (McGregor *et al*., 2002; Schwab *et al*., 2002; Olthof *et al*., 2005b). These effects of high doses of betaine and choline might counterbalance the potential beneficial effects of their homocysteine-lowering properties. However, the regular dietary intakes of betaine and choline are far lower than the doses used in intervention studies.

Intake of betaine from foods is estimated to be 200–400 mg/day (Shaw *et al*., 2004; Fischer *et al*., 2005). Main food sources of betaine are wheat bran, wheat germ, spinach, pretzels, shrimps and wheat bread (Zeisel *et al*., 2003). Choline is present in the human diet primarily as lecithin, which is the trivial name for phosphatidylcholine. Intake of choline moieties from foods is estimated at 400–600 mg/day (Shaw *et al*., 2004; Fischer *et al*., 2005). Main food sources of choline are beef liver, chicken liver, eggs, wheat germ, bacon, dried soybeans and pork (Zeisel *et al*., 2003).

Low folate intake is generally associated with an increased risk of CVD (Rimm *et al*., 1998; Voutilainen *et al*., 2001; Bazzano *et al*., 2002), and for stroke (Bazzano *et al*., 2002; He *et al*., 2004). The relationship between regular dietary intake of betaine and choline and the risk of CVD has not been investigated yet. Therefore we investigated the association between dietary intakes of folate, betaine and choline and the risk of CVD in 16 165 Dutch postmenopausal women of the Dutch PROSPECT–EPIC cohort (European Prospective study Into Cancer and nutrition), who were followed for 8.1 years. In addition, we investigated the associations between dietary intakes of folate, betaine and choline and plasma homocysteine concentrations and cholesterol concentrations in a subsample of 1,610 women (homocysteine in a subsample of 903 women).

Methods

Between 1993 and 1997, 17 357 women between 49 and 70 years were recruited among breast cancer screening participants in the PROSPECT–EPIC cohort, which is 1 of the 2 Dutch contributions to the European Prospective Investigation into Cancer and Nutrition (EPIC) (Boker *et al*., 2001).

Of the 17 357 women, 355 women who did not consent to linkage with vital status registries were excluded. Additionally, we excluded 117 women because of missing questionnaires, 92 women because they reported an energy intake of <500 or >6000 kcal/day and 628 women who reported a history of CVD before the baseline measurements, leaving 16 165 women available for analysis.

Baseline measurement

At baseline a general questionnaire containing questions on demographic characteristics, presence of chronic diseases, and risk factors for chronic diseases, such as hypertension, reproductive history, family history, smoking habits, drinking of alcohol and physical activity was administered. Systolic and diastolic blood pressures were measured in duplicate at the left arm with the subjects in sitting position after 10 min of rest with an automated and calibrated oscillomat (Bosch & Son, Jungingen, Germany). Subsequently, the mean systolic and diastolic blood pressure was calculated. Body height was measured to the nearest 0.5 cm with a wall mounted stadiometer (Lameris, Utrecht, The Netherlands). Body weight was measured in light indoor clothing without shoes to the nearest 0.5 kg with a floor scale (Seca, Atlanta, GA, USA). Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). We used a questionnaire for assessment of physical activity. This questionnaire has been validated in free living, apparently healthy people, aged 63–80 years (Voorrips *et al*., 1991). Classifications based on questionnaire activity scores showed Spearman's correlations of 0.78–0.73 with classifications obtained by repeated 24-h activity recalls and pedometer measurements, showing that the questionnaire provides a reliable and valid method for assessing physical activity in

this age-group. Also a blood sample was taken and stored under liquid nitrogen at −196°C.

Food frequency questionnaire (FFQ)

At the baseline visit of the PROSPECT study, each participant completes a FFQ. The FFQ was designed and carefully validated to estimate the intake of 178 food items in the year preceding enrollment (Ocke *et al*., 1997a, b). We used this FFQ to estimate the dietary intakes of folate, betaine and choline. The questionnaire contained colour photographs of 2–4 different-sized portions of 21 food items, which helped assessing the serving size. Subjects indicated their consumption frequency of each food item on a daily/weekly/monthly/yearly scale or as never consumed. For several food items, additional questions regarding consumption frequency of sub-items were asked. Questionnaires also included some blank-spaced questions, in which names of brands used could be filled in.

Calculation of dietary betaine, choline and folate intakes

The choline and betaine contents in individual foods were estimated from the available data of the USDA database with the choline and betaine contents of common US foods (Howe *et al*., 2004). For the choline contents, different types of choline are measured in the USDA-database that is, free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine and sphingomyelin. For this study we used the sum of all choline moieties to estimate choline intake.

We assigned betaine and choline contents to each food item in the food questionnaire of the PROSPECT study as follows. For food items of our questionnaire that occurred in the database of the USDA, we used the betaine and choline content data available. This was possible for fruit, vegetables and some meat products. For items in the questionnaire that did not have a direct correspondence to a food in the USDA database we estimated the choline and betaine contents of nutritionally comparable foods that were available. For example, for 'sunflower oil' choline and betaine values from 'olive oil' were assigned. Assignment of betaine and choline values to mixed dishes in our food frequency database, without a comparable mixed dish on the USDA database, was based on the proportional contribution of individual food components in the recipe. The recipe of the NEVO table (Dutch food composition table) (NEVO, 2001) was used. If there was no description of a recipe in the NEVO table then the recipe of a standard Dutch recipe book was used to decide on the individual components and their contribution to the mixed dish (Henderson *et al*., 1994). We computed women's average daily intakes of betaine and choline by calculating portion size and frequency of consumption of each food item. The folate intakes of the women in this study population were calculated by means of the Dutch National Food Database (NEVO, 2001).

Morbidity and mortality follow-up

Data on coronary heart disease (CHD) events and cerebrovascular accident (CVA) events morbidity data were obtained from the Dutch Centre for Health Care Information, which holds a standardized computerized register of hospital discharge diagnoses. All diagnoses were coded according to the International Classification of diseases, Ninth Revision, Clinical Modification (ICD-9). Follow-up was complete until 1January 2004. The database was linked to the cohort on the basis of birth date, gender, postal code and general practitioner with a validated probabilistic method (Herings *et al*., 1992).

Information on vital status was obtained through linkage with the municipal administration registries. Causes of death were obtained from the women's general practitioners.

For our analysis, CHD (ICD-9; 410–414,427.5) and CVA (ICD-9; 430–438), whichever came first, were the endpoints of interest. During the follow-up of 8.1 year in total, 717 women were diagnosed for first time with CVD; 493 with CHD and 224 with CVA.

Biochemical analysis

Between August and October 2000, serum and plasma samples of a randomly selected subsample of 1610 women in the cohort were retrieved from the liquid nitrogen. Serum total cholesterol was determined using an automated enzymatic procedure on a Vitros 250 (Johnson & Johnson, Rochester, NY, USA). Serum LDL- and high-density lipoprotein (HDL)-cholesterol were measured using a colorimetic assy on a Hitachi 904 (Johnson & Johnson, Rochester, NY, USA). All remaining samples were stored at −80°C. In November 2004, we selected a subsample of 903 women who has been fasting for at least approximately 2 h before blood sampling out of the first subsample of 1610 women. In the blood samples of these women, the total homocysteine concentrations (sum of all oxidized and reduced forms of homocysteine) were measured in plasma samples with the use of an high-performance liquid chromatography method with fluorescence detection (Ubbink *et al*., 1991). Within– and between run CVs were 3.1 and 5.9% respectively.

Data Analysis

Mean intakes and s.d. of folate, betaine and choline were calculated. To control for total energy intake, all nutrients were adjusted for total energy intake by using the regression residual method (Willett and Stampfer, 1986).

We counted for each participant the person-years of follow-up from the date of return of the questionnaire to the date of CVD diagnosis; or the end of follow-up at 1 January 2004. Cox-regression analysis was used to quantify the association of betaine, choline and folate intakes with total CVD and with the subtype CHD and CVA. Folate, betaine and choline intakes were categorized in quartiles, and hazard ratios were calculated for quartiles with the

lowest quartile of intake as the reference category. To study the relationship between folate, betaine and choline intakes and the risk on CVD we applied three models, that is an unadjusted model, a basic model that adjusted for conventional risk factors, and a fully adjusted model that took into account both conventional risk factors and nutritional factors. Linear regression analysis was used to assess the association of folate, betaine and choline intakes with plasma homocysteine, serum total cholesterol, LDL-cholesterol and HDL-cholesterol. For this, folate, betaine and choline intakes were categorized into quartiles, and differences in mean plasma homocysteine and serum cholesterol concentrations were calculated for the three upper quartiles of intake as compared to the lowest quartile of intake.

All analyses were conducted using SPSS for windows, Version 12.0.1.

Results

Baseline characteristics of the study population are shown in Table 1. Mean (±s.d.) betaine intake was 214±74 mg/day, mean choline intake was 300±51 mg/day and mean folate intake was 195±40 μg/day. In total 717 women were diagnosed with first time CVD; 493 women were diagnosed with CHD and 224 women with CVA during follow-up (Table 2).

In the unadjusted model and after adjustment for potential confounders, neither betaine nor folate intakes were associated with total CVD, CHD or cerebrovascular disease (Table 3). High choline intake tended to correspond with a higher risk of CHD, but this was only statistically significant in the unadjusted model. Choline intake was not associated with total CVD or cerebrovascular disease (Table 3).

Mean (±s.d.) concentrations of homocysteine, total cholesterol and HDL- and LDL-cholesterol were 12.0± 3.6 μmol/l ($n=903$ plasma samples), 5.8±1.0, 1.6±0.4 and 4.0±0.9 mmol/l ($n=1610$ serum samples), respectively. After adjustment for age, BMI and smoking, higher folate intake was statistically significantly associated with lower homocysteine levels (difference between fourth and first quartile –0.71±0.32 μmol/l, 95% CI −1.33; −0.08), but showed no relationship with lipid levels (Table 4).

Higher betaine intake was statistically significantly associated with lower HDL-cholesterol (difference between fourth and first quartile −0.06±0.03 mmol/l, 95% CI −0.11; −0.01), but there was no association with homocysteine concentrations (Table 4). Higher choline intake was statistically associated with lower homocysteine levels (difference between fourth and first quartile – 0.87±0.35 μmol/l, 95% CI −1.56; −0.19). Further the LDL-cholesterol concentration was higher among the second quartile of choline intake than the first (difference 0.14±0.07 μmol/l, 95% CI 0.01; 0.27), but the third and fourth quartiles were not different from the first quartile.

Table 1 Baseline characteristics of the study population of 16165 women

Variable	
	Mean±s.d.
Follow-up time, (months)	97±19.3
Age, (years)	57±6
BMI, (kg/ m^2)	26±4
Systolic blood pressure, (mm Hg)	133±20
Diastolic blood pressure, (mm Hg)	79±10
Physicial activity	6.9±5.0
	N (%)
Smoking status	
Smoking never	7046 (43.6)
Smoking past	5582 (34.5)
Smoking current <10	1648 (10.2)
Smoking current 11-20	1337 (8.3)
Smoking current >20	548 (3.4)
Hypertension	3082 (19.1)
Diabetes	434 (2.7)
Hypercholesterolaemia	788 (4.9)
Multi vitamin status	
Multivitamin, never	11753 (72.7)
Multivitamin, user	1146 (7.1)
Multivitamin, unknown	3266 (20.2)
Daily dietary intake[a]	Mean±s.d.
Betaine, (mg/day)	241±74
Choline, (mg/day)	300±51
Folic acid, (μg/day)	195±40
Energy, (kcal/day)	1798±436
Protein, (% of energy)	16.1±2.41
Carbohydrates, (% of energy)	44.7±6.4
Total fat, (% of energy)	35.6±5.5
Saturated fat, (g/day)	29±5
Monounsaturated fat, (g/day)	26±5
Polyunsaturated fat, (g/day)	13±4
Cholesterol, (mg/day)	199±54
Dietary fibre, (g/day)	22±4
Alcohol, (% of energy)	3.6±4.9
Vitamin B_2, (mg/day)	1.6±0.4
Vitamin B_6, (mg/day)	1.5±0.2
Vitamin B_{12}, (μg/day)	4.3±1.8

Abbreviations: BMI, body mass index.
[a]All dietary variables were adjusted for energy intake, except energy.

Discussion

We did not find an association between regular intake of betaine, choline or folate and CVD risk after adjustment for potential confounders in postmenopausal women. In a subsample of this population, we found that high choline and folate intakes, but not betaine intake, were associated with modestly lower homocysteine levels. Furthermore, we found that a high betaine intake was associated with slightly lower HDL cholesterol.

Before we interpret these findings, we will address internal validity of the study. First, the prospective design of our study. We assessed the average intake of dietary betaine, choline and folate before diagnosis of CVD, which

Table 2 Data on intake, follow-up time and number of cardiovascular events per quartile of energy-adjusted betaine, choline and folate intake

	Quartiles			
	1	*2*	*3*	*4*
Betaine				
Betaine range (mg/day)	$\times \leqslant 192$	$192 < \times \leqslant 234$	$234 < \times \leqslant 283$	$283 < \times$
Mean betaine intake (mg/day)	153	213	258	338
Average follow-up time, (months)	97	96	97	98
Total cardiovascular cases	188	172	178	179
CHD	127	116	124	126
CVA	61	56	54	53
Choline				
Choline range in (mg/day)	$\times \leqslant 266$	$266 < \times \leqslant 296$	$296 < \times \leqslant 329$	$329 < \times$
Mean choline intake (mg/day)	239	281	311	365
Average follow-up time, (months)	96	97	97	97
Total cardiovascular cases	166	158	188	205
CHD	108	101	127	157
CVA	58	58	61	48
Folate				
Folate range (μg/day)	$\times \leqslant 169$	$169 < \times \leqslant 191$	$191 < \times \leqslant 215$	$215 < \times$
Mean folate intake (μg/day)	151	180	202	246
Average follow-up time, (months)	97	98	97	97
Total cardiovascular cases	187	154	179	197
CHD	131	105	125	132
CVA	56	49	54	65

Abbreviations: CHD, coronary heart disease; CVA, cerebrovascular accident.

eliminates the recall bias that so often hampers interpretation of findings of case–control studies, as patients might recall their dietary habits differently than healthy controls. Second, the estimations of dietary intakes. The questionnaire was not validated to estimate dietary betaine, choline and folate intakes, but has been shown to adequately assess intake of energy, macronutrients, dietary fibre and retinol and included several specific foods that are rich in betaine, choline or folate (Ocke *et al*., 1997b). The relative validity for bread, which contains high amounts of betaine and folate, was good (Spearman's correlation coefficient 0.78), whereas for eggs, an important source of choline, the (Spearman's correlation coefficient was 0.43). For the most important sources of folate, that is milk and milk products, relative validity was good, and for vegetables it was adequate (Spearman's correlation coefficients 0.79, 0.31 respectively). Although the FFQ was not validated for B vitamins, the spearman correlations of the nutrients retinol, β-carotene, vitamin C and vitamin D are between 0.61 and 0.81. These correlation coefficients are all statistically significantly different from zero. Furthermore, the validity for the major food sources of B vitamins was also good, so we have no reason to assume that it would be different for B vitamins (Ocke *et al*., 1997a). Furthermore, our estimates of daily betaine, choline and folate intakes were well in line with other studies in western population (Melse-Boonstra *et al*., 2002; Shaw *et al*., 2004; Brink *et al*., 2005; Fischer *et al*., 2005; Larsson *et al*., 2005; Slow *et al*., 2005; Cho *et al*., 2006). Therefore we feel confident that the estimated intakes of betaine, choline and folate in our study are reliable. Nevertheless, the fact that our questionnaire was not specifically designed for measuring intakes of folate, betaine or choline may have led to an attenuation of the association between these nutritional compounds and risk of CVD.

The current evidence regarding folate intake and CVD risk from other prospective epidemiologic studies is generally in favour of a protective effect of folate on CVD. Most prospective studies found an inverse association between folate intake and risk of CVD (Rimm *et al*., 1998; Voutilainen *et al*., 2001; Bazzano *et al*., 2002), and for stroke (Bazzano *et al*., 2002; He *et al*., 2004), whereas one study did not (Al-Delaimy *et al*., 2004). This is sustained by prospective studies on folate status and risk of CVD (reviewed in Table 3 of Voutilainen *et al*. (2001). It is important to realize that the contrast in folate intake between the upper and lower categories in most studies is much larger than in our study, mainly owing to the fact that supplement use in our country is negligible.

Results from clinical intervention trials studying long-term effects of folic acid supplementation on CVD are inconclusive. Some clinical trials of homocysteine lowering through B-vitamin treatment support a cardioprotective effect (Schnyder *et al*., 2001, 2002), but others do not (Baker *et al*., 2002; Lange *et al*., 2004; Toole *et al*., 2004; Bonaa *et al*., 2006; HOPE 2 Investigators, 2006).

For betaine and choline, there are no other epidemiologic studies to compare our findings with. Our risk analyses suggested that a high choline intake may be associated with

Table 3 Hazard ratios for total cardiovascular events, CHD events and CVA events by quartiles of energy-adjusted betaine, choline and folate intake

	Hazard ratio (95% CI)			
Variable of model	*First quartile*	*Second quartile*	*Third quartile*	*Fourth quartile*
Betaine				
Total cardiovascular				
Unadjusted	1	0.88 (0.69; 1.13)	0.94 (0.74; 1.19)	0.96 (0.76; 1.22)
Basic model[a]	1	0.97 (0.76; 1.24)	0.97 (0.76; 1.24)	0.97 (0.76; 1.24)
Fully adjusted model[b]	1	0.94 (0.73; 1.21)	0.94 (0.72; 1.20)	0.90 (0.69; 1.17)
CHD				
Unadjusted	1	0.92 (0.71; 1.18)	0.97 (0.76; 1.24)	0.98 (0.77; 1.26)
Basic model[a]	1	1.00 (0.78; 1.29)	1.02 (0.79; 1.31)	1.00 (0.78; 1.28)
Fully adjusted model[b]	1	0.97 (0.75; 1.26)	0.98 (0.76; 1.28)	0.95 (0.72; 1.25)
CVA				
Unadjusted	1	0.92 (0.64; 1.33)	0.88 (0.61; 1.26)	0.86 (0.59; 1.24)
Basic model[a]	1	1.02 (0.71; 1.47)	0.92 (0.63; 1.33)	0.88 (0.61; 1.28)
Fully adjusted model[b]				
Choline	1	1.00 (0.69; 1.45)	0.90 (0.61; 1.33)	0.83 (0.55; 1.25)
Total cardiovascular				
Unadjusted	1	0.79 (0.61; 1.02)	1.04 (0.82; 1.33)	1.22 (0.97; 1.55)
Basic model[a]	1	0.78 (0.60; 1.01)	1.00 (0.78; 1.27)	1.08 (0.85; 1.38)
Fully adjusted model[c]	1	0.78 (0.58; 1.03)	0.99 (0.73; 1.34)	1.04 (0.71; 1.53)
CHD				
Unadjusted	1	0.93 (0.71; 1.21)	1.16 (0.90; 1.51)	1.44 (1.13; 1.84)*
Basic model[a]	1	0.91 (0.69; 1.20)	1.12 (0.86; 1.45)	1.26 (0.98; 1.62)
Fully adjusted model[c]	1	0.93 (0.69; 1.25)	1.14 (0.83; 1.56)	1.28 (0.86; 1.91)
CVA				
Unadjusted	1	0.97 (0.67; 1.40)	1.04 (0.73; 1.49)	0.82 (0.56; 1.20)
Basic model[a]	1	0.98 (0.68; 1.42)	1.01 (0.70; 1.46)	0.73 (0.49; 1.08)
Fully adjusted model[c]				
	1	0.95 (0.64; 1.43)	0.94 (0.60; 1.48)	0.61 (0.33; 1.13)
Folate				
Total cardiovascular				
Unadjusted	1	0.79 (0.61; 1.02)	0.95 (0.74; 1.20)	1.08 (0.85; 1.36)
Basic model[a]	1	0.82 (0.63; 1.06)	0.95 (0.74; 1.21)	1.06 (0.83; 1.34)
Fully adjusted model[d]	1	0.86 (0.64; 1.15)	1.05 (0.74; 1.49)	1.23 (0.75; 2.01)
CHD				
Unadjusted	1	0.79 (0.61; 1.03)	0.96 (0.75; 1.22)	1.00 (0.79; 1.28)
Basic model[a]	1	0.82 (0.63; 1.07)	0.96 (0.74; 1.23)	0.96 (0.75; 1.23)
Fully adjusted model[d]	1	0.83 (0.61; 1.12)	0.99 (0.69; 1.43)	1.05 (0.62; 1.79)
CVA				
Unadjusted	1	0.87 (0.59; 1.27)	0.97 (0.67; 1.41)	1.15 (0.81; 1.65)
Basic model[a]	1	0.91 (0.62; 1.33)	0.97 (0.67; 1.43)	1.16 (0.81; 1.68
Fully adjusted model[d]	1	0.99 (0.64; 1.54)	1.12 (0.66; 1.89)	1.38 (0.67; 2.81)

Abbreviations: CHD, coronary heart disease; CVA, cerebrovascular accident.

*$P<0.05$

[a]Adjusted for conventional risk factors, namely hypertension, cholesterolemia, mean systolic blood pressure, age, total physical activity, BMI (cat), smoking (never/ past/ current smoking 1–10, 11–20, and 20> cigarettes a day), and diabetes

[b]Adjusted for conventional risk factors and energy, protein intake (energy-adjusted), saturated fats intake (energy-adjusted), monounsaturated fats, polyunsaturated fats (energy-adjusted), alcohol intake (energy-adjusted), vitamin B_2 intake (energy-adjusted) vitamin B_6 intake (energy-adjusted) vitamin B_{12} intake (energy-adjusted), folate intake (energy-adjusted) and choline intake (energy-adjusted).

[c]Adjusted for conventional risk factors and energy, protein intake (energy-adjusted), saturated fats intake (energy-adjusted), monounsaturated fats, polyunsaturated fats (energy-adjusted), alcohol intake (energy-adjusted), vitamin B_2 intake (energy-adjusted) vitamin B_6 intake (energy-adjusted) vitamin B_{12} intake (energy-adjusted), betaine intake (energy-adjusted) and folate intake (energy-adjusted).

[d]Adjusted for conventional risk factors and intake of energy, proteins (energy-adjusted), saturated fats (energy-adjusted), monounsaturated fats, polyunsaturated fats (energy-adjusted), alcohol (energy-adjusted), vitamin B_2 (energy-adjusted) vitamin B_6 (energy-adjusted), vitamin B_{12} (energy-adjusted), betaine (energy-adjusted) and choline (energy-adjusted).

higher risk of CHD. This may be mediated by a triacylglycerol-raising effect, as suggested by a previous experiment (Olthof *et al*., 2005b).

We did not find an association between betaine and homocysteine concentrations, whereas intakes of choline and folate were only weakly inversely associated with concentrations of plasma homocysteine. This was probably a consequence of the narrow ranges of intake. Hence, the homocysteine differences between the upper and lower quartiles were most likely too small to detect a difference

Table 4 The association of dietary intakes of betaine, choline and folate with plasma levels of homocysteine ($n=903$) and total cholesterol, HDL-cholesterol and LDL-cholesterol ($n=1610$)

	Differences in mean concentration (CI 95%) between each quartile and the lowest quartile			
	Homocysteine (imol/l)	*Total –cholesterol (mmol/l)*	*HDL-cholesterol (mmol/l)*	*LDL-cholesterol (mmol/l)*
Betaine[a]				
First quartile	Reference	Reference	Reference	Reference
Second quartile	−0.49 (−1.15; 0.17)	−0.13 (−0.27; 0.00)	−0.02 (−0.07; 0.04)	−0.11 (−0.24; 0.02)
Third quartile	−0.45 (−1.12; 0.21)	−0.09 (−0.23; 0.04)	−0.05 (−0.10; 0.01)	−0.11 (−0.24; 0.02)
Fourth quartile	−0.32 (−0.98; 0.34)	−0.07 (−0.21; 0.06)	−0.06 (−0.11; −0.01)*	−0.02 (−0.14; 0.12)
Choline[a]				
First quartile	Reference	Reference	Reference	Reference
Second quartile	−0.08 (−0.75; 0.60)	0.11 (−0.03; 0.25)	−0.03 (−0.08; 0.02)	0.14 (0.01; 0.27)*
Third quartile	−0.53 (−1.21; 0.14)	0.04 (−0.10; 0.17)	−0.02 (−0.08; 0.03)	0.06 (−0.07; 0.19)
Fourth quartile	−0.87 (−1.56; −0.19)*	0.11 (−0.03; 0.25)	−0.03 (−0.09; 0.03)	0.11 (−0.02; 0.24)
Folate[a]				
First quartile	Reference	Reference	Reference	Reference
Second quartile	−0.14 (−0.74; 0.46)	0.01 (−0.12; 0.13)	−0.04 (−0.09; 0.01)	0.03 (−0.09; 0.15)
Third quartile	−0.62 (−1.34; 0.11)	−0.06 (−0.21; 0.08)	−0.05 (−0.11; 0.01)	−0.03 (−0.17; 0.11)
Fourth quartile	−0.71 (−1.33; −0.08)*	0.01 (−0.12; 0.13)	−0.04 (−0.09; 0.01)	0.00 (−0.12; 0.12)

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein
*$P<0.05$.
[a]Adjusted for age, BMI and smoking.

in disease risk between the quartiles, if high homocysteine is truly a cause of CVD, of course. Doses of betaine used in the supplementation studies that were able to affect homocysteine levels are between 1.5 and 6.0 g/day, which is far higher than the regular dietary intake (Schwab *et al*., 2002; Olthof *et al*., 2003; Steenge *et al*., 2003). This also holds for choline and to a lesser extent for folate acid. In addition the blood sampling in this study was not optimized for homocysteine analyses. Subjects were not completely fasted, blood was not kept cool after sampling and plasma was not prepared within an hour after sampling. All these factors may have contributed to a large variation in homocysteine values, whereby the relationships between betaine, choline and folate intakes and homocysteine concentrations are weakened. Cho *et al*. (2006) also examined the relationships between choline and betaine and plasma homocysteine in 1960 subjects. They found that higher intakes of dietary choline and betaine were related to lower total homocysteine concentration. However the range of betaine and choline intake in their study was somewhat broader than in our study.

We did not find any significant associations between intake of betaine, choline and folate intakes and total cholesterol levels. Women in the fourth quartile of betaine intake had slightly lower HDL-cholesterol levels than women in the first quartile, but they did not have a higher risk of CVD. Intervention studies suggest that high doses of betaine and choline increase blood lipids (McGregor *et al*., 2002; Schwab *et al*., 2002; Olthof *et al*., 2005b). However, the dietary intakes of betaine and choline found in the current study are far below the doses used in intervention studies. Therefore, we conclude that intakes of betaine and choline in the dietary range are probably not strongly related to increased lipid concentrations. However, high intakes of betaine or choline, for example, with supplements, may increase lipid levels (Olthof *et al*., 2005b).

Conclusion

Regular dietary intakes of betaine, choline and folate were not associated with CVD risk in postmenopausal Dutch women. In line with this, associations of these nutritional compounds with homocysteine and lipids were absent or small. However, there may of course be an effect of high doses of these compounds, that is, beyond regular dietary intakes, on CVD risk.

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Conflict of interest

The authors declare no conflict of interest.

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