Dietary Betaine Intake and Risk of Mortality in Patients with Coronary Artery Disease: The Prospective Guangdong Coronary Artery Disease Cohort

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ABSTRACT
This study is designed to explore the association between dietary betaine intake and risk of all-cause and cardiovascular death in patients with CAD. In this cohort study, 1292 patients with CAD were followed-up for a median of 9.2 years. Baseline dietary betaine intake was collected using a paper-based semi-quantitative food frequency questionnaire (FFQ) and assessed according to the US Department of Agriculture (USDA) Database and the data of betaine in common foods. Cox proportional hazards regression models were used to analyze the association between dietary betaine intake and risks of all-cause and cardiovascular mortality. During the follow-up periods, 259 deaths recorded in 1292 participants, of which 167 died of cardiovascular diseases. Patients in the highest tertile of dietary betaine intake had a lower risk of all-cause ($P=0.007$) and cardiovascular death ($P<0.001$) than those in the lowest tertile after adjusting for age and sex, traditional cardiovascular risk factors and other potential confounders. After further adjusting for plasma methionine metabolites and vitamins, HRs across tertiles of dietary betaine intake were 1.00, 0.84 and 0.72 for all-cause mortality ($P$ for trend=$0.124$), and 1.00, 0.77 and 0.55 for cardiovascular mortality ($P$ for trend=$0.021$). Higher dietary betaine intake was associated with a decreased risk of cardiovascular death after fully adjustment for cardiovascular risk factors, other potential confounders and plasma methionine metabolites and vitamins. However, the association between dietary betaine intake and risk of all-cause mortality was not statistically significant after further adjusting for plasma methionine metabolites and vitamins.

Key words: dietary betaine; coronary artery disease; cardiovascular; mortality
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Introduction

Cardiovascular diseases (CVD) remain the leading cause of premature death and rising medical costs globally\(^1\). In 2019, the number of prevalent cases of CVD was about 523 million and the number of CVD deaths was 18.6 million, showing a stable increasing trend compared with 1990\(^2\). CVDs involve disorders of the heart and blood vessels and include coronary artery diseases (CAD), stroke, heart failure, etc.\(^3\) CAD is the most common type of CVD\(^1,4\), and the main drivers of CAD include cardiometabolic, environmental, behavioral, and social risk factors\(^2\). There are some modifiable risk factors, such as high systolic blood pressure, high fasting plasma glucose, high low-density lipoprotein cholesterol, high body mass index (BMI), impaired kidney function, ambient and household air pollution, tobacco, dietary risks, low physical activity\(^2,5\). It is estimated that more than 90% of CAD could be prevented through early intervention\(^6\) and then could reduce CAD mortality and burden\(^5\).

Methionine (Met) is a unique amino acid and also proteogenic amino acid necessary of the canonical 20 amino acids building proteins, which contains sulfur and can be transformed into other sulfur-containing molecules in vivo\(^7,8\). Methionine is the initiating amino acid and plays a vital role as an initiator of protein synthesis in almost all prokaryotes and eukaryotes\(^9\). In body, methionine can not only construct proteins, but also have other special functions, such as modifying DNA, regulating methylation reaction and maintaining proper functioning of the cells\(^10,11\). In addition, methionine metabolism is closely related to various metabolic pathways and plays an important role through its metabolism. It has been reported that dysregulation of methionine metabolism is related to a variety of diseases, such as obese\(^12\), cancer\(^13\), CVD\(^14\), etc. The first step of methionine metabolism is the biosynthesis of SAM through methionine adenosyltransferase (MAT) catalyzing methionine and ATP. Then SAM donates its methyl group to substrates in the methylation process and generates SAH through methyltransferases\(^15\). The conversion of SAH to homocysteine (Hcy) and adenosine is a reversible hydrolysis reaction, which is catalyzed by S-denosylhomocysteine hydrolase (SAHH/AHCY). Homocysteine can be remethylated by the methionine synthase (MS) with 5-methyltetrahydrofolate as a methyl donor or betaine homocysteine methyltransferase (BHMT) with betaine as a methyl donor to form methionine\(^9\). SAH and betaine are two important molecules in the methionine cycle. SAH has varying degrees of inhibition on different methyltransferases and is a known product inhibitor of SAM-dependent methylation reactions, so low levels of SAH are critical to maintaining normal methylation in cells. Betaine is distributed widely in many foods and sever as a methyl donor which may affect the re-methylation of methionine\(^16\). The concentrations of betaine in wheat bran, wheat germ, spinach pretzels, shrimp and wheat bread are relatively high\(^17\).
Choline is the metabolic precursor of betaine, which can be easily obtained from beef liver, chicken liver, eggs, wheat germ, bacon, dried soybeans and pork. Inadequate dietary intake of methyl donor food leads to hypomethylation of many metabolic pathways, leading to various diseases, such as diabetes, cardiovascular disease and metabolic syndrome. Previous studies have shown that dietary betaine intake may reverse alcoholic fatty liver and protect against coronary artery disease. However, some literatures have indicated that dietary betaine metabolite trimethylamine N-oxide (TMAO) is related with the risk of CVD. The contradictory results of these studies raise the question of the role of dietary betaine in the risk of CVD. Epidemiologic evidence for dietary betaine and mortality is limited, and there are few studies based on the Chinese population. In our study, we prospectively evaluated the relationship of dietary betaine intake with the risk of mortality in patients with CAD based on Chinese population.

Methods
Study Population

The data for this study are from the Guangdong Coronary Artery Disease Cohort, which is a prospective observational cohort study investigating the influence of environmental, social, and genetic factors on the progression and prognosis of CAD. Participates were recruited between October 2008 and December 2011. Patients aged 40 to 85 years were enrolled from the cardiology departments of 3 major hospitals (General Hospital of Guangzhou Military Command of People’s Liberation Army, the First Affiliated Hospital and Second Affiliated Hospital of Sun Yat-sen University) in Guangzhou in South China (23° 16’ north latitude). This study was approved by the Ethical Committee of Sun Yat-sen University. Written informed consent was provided by all participants at the time of enrollment. All of the protocols adhered to institutional guidelines and to the Helsinki Declaration. The rationale and design of this cohort study, inclusion and exclusion criteria, methods, and definitions refer to the previous studies. For this analysis, additional exclusion criteria included participants taking supplements containing choline or betaine, and participants who were pregnant or breastfeeding at the time of enrollment. According to WHO 1999/2000 guidelines, a total of 1977 patients underwent coronary angiography were diagnosed with CAD. After excluding 389 participants with missing baseline data or lacking sufficient plasma samples and 296 patients with missing baseline FFQ data, 1292 patients were included for analysis in the present study (Supplementary Figure 1). Our cohort study was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline (S1 STROBE Checklist).
Analysis of Coronary Angiography

Coronary angiography was performed in all of the participants with the standard Judkins technique through the femoral artery or brachial artery and scored for luminal narrowing using a modified American Heart Association/American College of Cardiology classification of the coronaries\(^{(29)}\). The angiography was interpreted by at least 2 independent cardiologists who were blinded to the patient's risk factors. CAD patients are defined in three levels, namely Patients with CAD were defined in three levels, namely mild CAD (visible plaque resulting in >20% but <50% luminal narrowing stenosis), moderate (≥50%–<70% stenosis), and obstructive CAD (≥50% stenosis in the left main coronary artery, or ≥70% in any other coronary artery, or both). Patients with obstructive CAD were further categorized by the number of diseased vessels, namely a single, double, or triple-vessel distribution. Taken together, the Gensini score was used to comprehensively evaluate the extent of CAD in current study\(^{(30)}\). The methods of coronary angiography analysis have been presented in our previous articles\(^{(31)}\).

Dietary assessment

The dietary intake of all participants was collected using a paper-based semi-quantitative food frequency questionnaire (FFQ), which was used to assess the usual consumption of major nutrients and food groups\(^{(32)}\). The FFQ, which included 8 food groups with a total of 81 food items, had been verified to have satisfactory reproducibility and reasonable validity in previous study\(^{(32)}\). The frequency and portion-size data of the participants were calculated using the China Food Composition Table 2004\(^{(33)}\). The total amount of dietary betaine intake was assessed according to the US Department of Agriculture (USDA) Database\(^{(34)}\) and the data of betaine in common foods\(^{(17)}\). The residual method proposed by Willett & Stampfer was used to adjust the nutrient intake in the total energy intake\(^{(35)}\).

Baseline Measurements and Biochemical Analyses

General information about personal basic information, living habits, dietary, leisure-time physical activity, clinical data and measurement indexes, use of medications and so on was collected by using standardized questionnaires during clinical face-to-face interviews. Menopause was defined as the absence of menstruation for ≥12 months and women ≥55 years old without menopausal information were considered postmenopausal\(^{(36)}\). Medical records were reviewed by medical staff, and then the questionnaires were checked by a trained interviewer for missing data and completeness before the data were entered into a database. Anthropometric measurements methods, information
presentation form and biochemical analyses have been described in our previous studies\(^{31}\). The inter-assay and intra-assay coefficients of variations of plasma lipid levels, creatinine, fasting blood glucose, plasma total cysteine (tCys) and tHcy, serum vitamin B\(_{12}\) were ≤8.6%.

**Plasma SAM and SAH Measurements**

Plasma SAM and SAH are more prone to degradation without any treatment. Therefore, the collected plasma samples should be immediately aliquoted, acidified\(^{37}\) and stored at -80°C until analyzed. The treated samples can be stored at -80°C for at least one month. Based on HPLC-MS technology, plasma SAH and SAM were measured by stable-isotope dilution which allows sensitive and rapid measurement of the two molecular\(^{37}\).

**Outcomes of Follow-up**

The main outcomes of this study were all-cause mortality and cardiovascular mortality. The final date of follow-up was December 30, 2019. Annual follow-up information was collected and confirmed during a median follow-up of 9.2 years (interquartile range: 8.5-10.2 years). Death certificates were coded by nosologists according to the *International Classification of Diseases*. Cardiovascular mortality was defined as death attributable to an ischemic cardiovascular cause (including fatal myocardial infarction, stroke, and peripheral arterial disease) or sudden death due to an unknown but presumed cardiovascular cause in high-risk patient according to the *International Classification of Diseases Tenth Revision codes I00-I99*.

**Statistical Analyses**

Participants were categorized according to the tertiles of dietary betaine intake. Continuous variables for baseline characteristics are presented as means ± SD or median with interquartile range and categorical variables of baseline characteristics are presented as counts and percentages (%). Differences between groups were compared by using 1-way ANOVA and the \(\chi^2\) test. The relationships between dietary betaine intake and plasma concentrations of methionine metabolites, including SAH, SAM, SAM/SAH, tHcy, tCys, folate and vitamin B\(_{12}\) based on partial correlational analysis. The cumulative event plot for all-cause mortality and cardiovascular mortality according to the tertiles of dietary betaine intake was estimated by using the Kaplan-Meier method and \(p\) values were compared by using the log-rank test. Betaine was not only obtained from diet, but also from the oxidation of dietary choline in vivo\(^{38}\). Therefore, as dietary choline might affect the plasma level or dietary intake of betaine, models would be adjusted for dietary choline intake. The hazard ratio (HR)
and 95% confidence interval (CI) of the outcomes of all-cause mortality and cardiovascular mortality according to increasing dietary betaine intake was calculated with Cox proportional hazards models adjusted for traditional cardiovascular risk factors (Model 1) and traditional cardiovascular risk factors plus metabolites related to methionine cycle (Model 2). Model 1 was adjusted for age, sex, body mass index, smoking status, alcohol drinker, hypertension, diabetes mellitus, physical activity, family history of CAD, systolic blood pressure, gensini score, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, use or nonuse of statins, aspirin, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, β-blockers, dietary energy intake, dietary protein intake, and dietary choline intake. Model 2 was adjusted for variables in Model 1 plus SAH, SAM, tHcy, tCys, folate, vitamin B-12. The restricted cubic splines were used to estimate nonlinear dose-response relationship between dietary betaine intake and HRs for the outcomes. Additionally, we performed a further analysis on subgroups stratified by baseline covariates including age, sex, and other traditional CVD risk factors with risk of all-cause mortality and cardiovascular mortality. Using the group categorized according to the tertiles of dietary betaine intake, we calculated crude and three adjusted HR with 95% CI.

All statistical analyses were performed with SPSS 25.0 (IBM SPSS Inc, Chicago, IL) and the restricted cubic splines were plotted by R language (Version R-4.0.3). A two-sided P value <0.05 was considered statistically significant.

**Results**

**Baseline Characteristics**

After excluding the patients without enough plasma samples or with missing data, a total of 1292 participants were included in this analysis. At the baseline, the average age was 64.1±11.5 years, and 68% of the participants were male. The cutoff points of tertiles of dietary betaine intake were 212 and 257 mg/d. Table 1 presents the baseline characteristics of all the 1292 patients according to the tertiles of dietary betaine intake. Among tertiles, there were no significant difference in gender, age and BMI. Participants in the first and second tertiles of dietary betaine intake were more likely to be higher prevalence of hypertension and systolic blood pressure than those in the third tertile (P<0.05). According to tertiles of dietary betaine intake, participants with a higher dietary betaine intake had lower level of triglycerides. Dietary choline intake was positively correlated with the dietary betaine intake.
Association of Dietary Betaine Intake with Plasma Concentrations of Methionine Metabolites and Vitamins

Table 2 showed the relationship of dietary betaine intake with plasma concentrations of methionine metabolites and vitamins according to tertiles of dietary betaine intake. For the methionine metabolites in plasma, participants in higher tertiles of dietary betaine intake had a significantly lower level of SAH and tHcy, but higher level of SAM and higher ratio of SAM/SAH. Plasma tCys concentration did not differ significantly among the three tertiles of dietary betaine intake, nor did the plasma vitamins concentrations (folate and vitamin B₁₂). And results of the partial correlation analysis of the associations between dietary betaine intake and plasma concentrations of methionine metabolites and vitamins were showed in Figure 1 and Supplementary Table 1., dietary betaine intake was inversely associated with the level of plasma SAH (r= -0.194, P<0.001, Figure 1A) and tHcy (r= -0.167, P<0.001, Figure 1C), but positively associated with the level of plasma SAM (r=0.147, P<0.001, Figure 1B) and SAM/SAH ratio (r= 0.240, P<0.001), which was the same as in univariate analysis. There was no significant correlation between dietary betaine intake and plasma tCys, folate and vitamin B₁₂ in both univariate analysis and multivariate analysis.

Association of Dietary Betaine Intake with Risk of Mortality

During a median follow-up of 9.2 years (interquartile range: 8.5-10.2 years), there were 259 deaths (20.0%) recorded in 1292 participants, of which 167 (12.9%) died of cardiovascular diseases. Kaplan-Meier analysis showed that dietary betaine intake as tertiles was significantly inversely correlated with all-cause mortality risk (log-rank, P<0.001, Figure 2A) and cardiovascular mortality risk (log-rank, P<0.001, Figure 2B). The unadjusted HRs for each 1 SD increase in daily betaine intake were 0.75 (95% CI, 0.65-0.85, P<0.001) for all-cause mortality, and 0.62 (95% CI, 0.53-0.74, P<0.001) for cardiovascular mortality, respectively (Table 3). Compared with patients in the lowest tertile, patients in the highest tertile of dietary betaine intake had a lower risk of all-cause death (adjusted HR, 0.52; 95% CI, 0.38-0.72; P<0.001) and cardiovascular death (adjusted HR, 0.37; 95% CI, 0.24-0.56; P<0.001) in age- and sex-adjusted analysis (Table 3). Assessment of continuous values of daily betaine intake showed that 1 SD increase in the daily betaine intake was associated with a 18% lower risk of all-cause mortality (adjusted HR, 0.82; 95% CI, 0.72-0.94; P=0.007) and a 30% smaller risk of cardiovascular death (adjusted HR, 0.70; 95% CI, 0.58-0.82; P<0.001) in the multivariable-adjusted model 1 analysis (Table 3). In the multivariable-adjusted model 2, HRs across tertiles of dietary betaine intake were 1.00, 0.84 and 0.72 for all-cause mortality (P for trend=0.124), and 1.00, 0.77 and 0.55 for cardiovascular mortality (P for trend=0.021) (Table 3). The restricted cubic splines further showed a dose-response association between dietary betaine intake and...
all-cause or cardiovascular mortality of multivariable-adjusted model 1 and multivariable-adjusted model 2 analysis, although the inverse association trends were not significant \( (P>0.05) \) (Figure 3). We further analyzed the relationship between daily betaine intake and all-cause and cardiovascular mortality based on stratified baseline characteristics including age, sex, BMI, smoking status, alcohol drinking, hypertension and diabetes mellitus. However, among the above parameters, there was no significant interaction between daily betaine intake and all-cause and cardiovascular mortality (Supplementary Table 2).

**Discussion**

In our prospective cohort study, we explored the relationship between dietary betaine intake and the risks of all-cause mortality and cardiovascular mortality in patients with CAD. Daily betaine intake was negatively correlated with the risks of all-cause and cardiovascular mortality, but the association between dietary betaine intake and risk of all-cause mortality was not statistically significant after further adjusting for plasma methionine metabolites and vitamins.

Baseline characteristics analysis showed that patients with higher daily betaine intake were more likely to be lower prevalence of hypertension and systolic blood pressure and showed lower level of triglycerides, but had a higher level of dietary choline intake. High blood pressure caused blood vessels to narrow, and then blood flowed to the heart may slow down or even stop, making it difficult for the heart to pump blood. Hypertension was the risk factor for premature cardiovascular disease\(^{(39,40)}\) and almost half of cardiovascular events was related to hypertension globally\(^{(41)}\). Animal experiments showed that betaine could improve hypertension by inhibiting inflammatory response\(^{(42)}\). In previous cohort study, researcher found that betaine possibly contributed to blood pressure regulation in female patients\(^{(43)}\). SBPs were associated with the CVD mortality risks and hypertension and higher systolic blood pressure could significantly increase the CVD mortality risk\(^{(44)}\). In Chinese adults, blood pressure was independent association with the risk of CVD and systolic blood pressure was predictor of CVD risk\(^{(45)}\). Most of the plasma triglycerides were packed in lipoprotein particles (chylomicrons). Elevated triglycerides-rich remnant lipoproteins promoted responsible for atherosclerosis formation\(^{(46)}\). Elevated plasma triglyceride levels were strongly associated with smaller size of LDL-particle, which had a powerful atherogenic effect\(^{(47)}\). Triglyceride could stimulate atherosclerosis by producing pro-inflammatory cytokines and impairment of fibrinolysis\(^{(48)}\). Population-based prospective studies showed that increased plasma triglyceride level was associated with an increase incident cardiovascular disease\(^{(49,50)}\) and risk of all-cause mortality\(^{(51)}\). In general, the risk of cardiovascular disease was associated with the
prevalence of hypertension and systolic blood pressure and higher level of triglycerides, which could be improved by increased betaine intake, thereby reducing the risk and mortality of cardiovascular disease.

For the methionine metabolites in plasma, dietary betaine intake was positively correlated with the levels of plasma SAM and SAM/SAH ratio and negatively associated with the concentrations of plasma SAH and tHcy. Betaine is distributed widely in many foods and severed as a methyl donor, which may affect the re-methylation of methionine and in turn affect the level of metabolites in the methionine cycle. Betaine could methylate homocysteine and decrease the level of homocysteine released by the liver\textsuperscript{(52)}. In our cohort study, we also found that the increase of betaine intake was inversely correlated with the level of tHcy. Homocysteine had adverse effects on vascular endothelial cells and smooth muscle cells, further lead to inflammatory reaction and promoted the formation of atherosclerosis\textsuperscript{(53)}. The increase of daily betaine intake can increase the concentration of SAM in plasma. Other studies had shown that betaine treatment could increase the level of plasma SAM\textsuperscript{(54)} and there was a strong positive correlation between plasma betaine and SAM\textsuperscript{(55)}. As a methylation donor, SAM could regulate the methylation reaction of the body. Administration of exogenous SAM to mice could change the inflammatory process, reduce oxidative stress\textsuperscript{(56)} and prevent endothelial dysfunction\textsuperscript{(57)}, which were all related to the occurrence of cardiovascular diseases. The results of our cohort study showed that the level of plasma SAM was negatively correlated with the risk of death\textsuperscript{(31)}. In this study, we found that higher intake of betaine was correlated with a decrease in plasma SAH levels, which was consistent with other reported studies\textsuperscript{(52)}. On the one hand, SAH could disrupt the DNA methylation\textsuperscript{(58)} by inhibiting the expression of DNA methyltransferase (DNMT1) and then promoted oxidative stress and induced vascular endothelial dysfunction via activating the expression of p66shc gene\textsuperscript{(59)}. The activation of oxidative stress could further induce the proliferation and migration of vascular smooth muscle cells to promote atherogenesis\textsuperscript{(60)}. Moreover, SAH induced macrophage apoptosis and accelerated the formation of atherosclerosis by modulating histone methylation\textsuperscript{(61)}. Previous studies had shown that elevated plasma SAH concentrations were associated with an increased risk of cardiovascular disease and atherosclerosis\textsuperscript{(24)}. Betaine supplementation were effective in increasing the ratio of SAM:SAH in hepatocytes\textsuperscript{(62)}. The decrease in the intracellular SAM:SAH ratio inhibited the activity of SAM-dependent methyltransferase\textsuperscript{(52)} and affected the normal methylation reaction, which played an important role in the regulation of atherosclerosis\textsuperscript{(63)}. On the whole, the increase of tHcy and SAH and the decrease of SAM and SAM/SAH ratio were related to the risk of cardiovascular disease, and betaine intake might affect the change of their levels and reduced the risk of cardiovascular disease.
Our results indicated that daily betaine intake was inversely correlated with and the risk of all-cause and cardiovascular death. After adjustment for cardiovascular risk factors and other potential confounders, the association between dietary betaine intake and risk of all-cause and cardiovascular death was still statistically significant. Higher dietary betaine intake was associated with a decreased risk all-cause and cardiovascular death after fully adjustment for cardiovascular risk factors, other potential confounders and plasma methionine metabolites and vitamins. However, the trend for all-cause mortality was not statistically significant ($P=0.124$) after adjustment for plasma methionine metabolites and vitamins. This may be due to that dietary betaine participates in the metabolism of methionine and affects the level of intermediate metabolites, such as SAH, SAM, tHcy, etc. These metabolites had an impact on health and death risk, after adjusting for these factors, weakening the predictability of betaine. However, our results were not completely consistent with previous studies. Betaine supplementation of mouse during the lactation could increase the content of betaine in breast milk, which could improve glucose homeostasis and led to reduce lower adiposity of the mouse offspring, while betaine supplementation could increase the Akkermansia abundance in the gut, so as to improve the long-term metabolic health of human offspring. In a cohort study of Japanese population, betaine intake was negatively associated with the risk of death from coronary heart disease mortality but not inversely correlated with the risk of mortality from stroke in men, but dietary betaine intake was not associated with the risk of mortality from stroke and coronary heart disease in women. There was a negative relationship between higher betaine consumption and all-cause and breast cancer mortality in an observational population-based Long Island Breast Cancer study. In a 10.6 year follow-up study with 2606 adults, the researchers found that the increase of dietary betaine has no relationship with the incidence of CVD. But some studies showed that increased intake of food containing betaine could increase the plasma TMAO levels and then increase the risk of CVD mortality. This inconsistency may be due to different populations, different health status, follow-up time and so on. The subjects in our cohort study were patients diagnosed with CAD, which was different with previous studies. Therefore, dietary betaine intake had certain prerequisites for predicting the risk of cardiovascular mortality and the risk of all-cause mortality.

**Limitations**

Some limitations of this study must also be acknowledged. First, we use self-reported FFQ to record dietary intake of foods which may lead to bias in their estimates of usual food consumption. Hence, the residual confounding factor that over or under report of dietary intake might affect the
evaluation of dietary betaine intake was not completely excluded. However, over or under report of dietary intake was associated with age, sex and BMI\(^{(69)}\). In this study, the distribution of age, sex and BMI were not significant difference between the tertiles of dietary betaine intake. So, this confounding factor might be partially balanced among the tertiles of dietary betaine intake. Second, we just collect the dietary intake of betaine at baseline and it may be changed over the follow-up. Third, other residual confounding cannot completely be ruled out even after carefully adjusting for possible confounders in our studies. Fourth, although we did not measure plasma choline and betaine levels, previous researches have shown that serum betaine level was positively associated with dietary betaine intake\(^{(38,70)}\) and intake of foods with high choline content could double plasma choline levels\(^{(38,71)}\). Therefore, it can be assumed that dietary intakes of betaine and choline may approximately reflect the serum status of betaine and choline in individuals. Fifth, subjects in the studies are all patients with CAD in China and the results are not applicable to other ethnicities. And last, the observational nature of our study makes it impossible to estimate the causality between dietary betaine intake and mortality.

**Conclusions**

Daily betaine intake was inversely correlated with the risk of all-cause and cardiovascular death. After adjustment for cardiovascular risk factors and other potential confounders, the association between dietary betaine intake and risk of all-cause and cardiovascular death was still statistically significant. Higher dietary betaine intake was associated with a decreased risk of cardiovascular death after fully adjustment for cardiovascular risk factors, other potential confounders and plasma methionine metabolites and vitamins. However, the trend for all-cause mortality was not statistically significant after adjusting for plasma methionine metabolites and vitamins.

**Acknowledgments**

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Conflict of Interest statement: None of the authors had a conflict of interest.

Data Share Statement: Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval, payment.

Abbreviations: CAD, coronary artery disease; CVD, cardiovascular disease; BMI, Body mass index; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; tCys, total cysteine; tHcy, total homocysteine; MAT, adenosyltransferase; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HHcy, hyperhomocysteinemia; HPLC, high-performance liquid chromatography; FFQ, food frequency questionnaire.
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Table 1. Baseline Characteristics of CAD Patients by Tertiles of Dietary Betaine Intake

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Tertiles of Dietary Betaine Intake*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 1 (n=430)</td>
<td>Tertile 2 (n=431)</td>
</tr>
<tr>
<td>Age, y</td>
<td>64.1 ± 11.8</td>
<td>64.6 ± 11.4</td>
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<tr>
<td>Male, n (%)</td>
<td>290 (67.4)</td>
<td>300 (69.6)</td>
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<tr>
<td>Postmenopausal female, n (%)</td>
<td>132 (30.7)</td>
<td>119 (27.6)</td>
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<tr>
<td>BMI, kg/m²</td>
<td>24.1 ± 3.4</td>
<td>23.9 ± 3.2</td>
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<tr>
<td>Waist circumference, cm</td>
<td>89.1 ± 9.6</td>
<td>89.4 ± 9.9</td>
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<tr>
<td>Current smoking, n (%)</td>
<td>175 (40.7)</td>
<td>152 (35.3)</td>
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<td>Secondhand smoking of non-smokers, n (%)</td>
<td>182 (42.3)</td>
<td>200 (46.4)</td>
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<td>Alcohol drinker, n (%)</td>
<td>121 (28.1)</td>
<td>104 (24.1)</td>
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<td>Hypertension, n (%)</td>
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<td>257 (59.6)</td>
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<td>Diabetes mellitus, n (%)</td>
<td>136 (31.6)</td>
<td>134 (31.1)</td>
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<tr>
<td>Family history of CAD, n (%)</td>
<td>33 (7.7)</td>
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<td>Physical activity &lt;4.0 h/wk, n (%)</td>
<td>176 (40.9)</td>
<td>182 (42.2)</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>135±22</td>
<td>136±23</td>
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<td>Diastolic blood pressure, mm Hg</td>
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<td>Statin, n (%)</td>
<td>145 (33.7)</td>
<td>142 (32.9)</td>
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<td>Aspirin, n (%)</td>
<td>137 (31.9)</td>
<td>151 (35.0)</td>
</tr>
<tr>
<td>ACE inhibitor or ARB, n (%)</td>
<td>131 (30.5)</td>
<td>136 (31.6)</td>
</tr>
<tr>
<td>β blocker, n (%)</td>
<td>184 (42.8)</td>
<td>185 (42.9)</td>
</tr>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.63±1.10</td>
<td>4.66±1.10</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.95±1.33</td>
<td>1.82±1.09</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.07±0.29</td>
<td>1.07±0.26</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.95±0.93</td>
<td>3.03±1.04</td>
</tr>
<tr>
<td>Gensini score</td>
<td>23.5 (9, 47)</td>
<td>25 (10, 50)</td>
</tr>
<tr>
<td>Dietary energy intake (MJ/d)</td>
<td>8.59±3.73</td>
<td>8.86±3.76</td>
</tr>
<tr>
<td>Dietary protein intake (g/d)*</td>
<td>73.8±14.0</td>
<td>73.5±12.6</td>
</tr>
<tr>
<td>Dietary choline intake (mg/d)*</td>
<td>273.7±69.1</td>
<td>283.1±73.0</td>
</tr>
</tbody>
</table>

1 Values expressed as mean ± SD values, median (interquartile range) values, and percentage (%) unless otherwise stated. Significance tests for comparisons by tertiles of dietary betaine intake based on analysis of variance for continuous variables and Pearson χ² test for categorical variables. The cutoff points of tertiles of dietary betaine intake were 212 and 257 mg/d. BMI, body mass index; CAD, coronary artery disease; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

* Nutrient intake was adjusted for energy intake using the residual method.
Table 2. Plasma concentrations of methionine metabolites and vitamins in Patients with CAD by Tertiles of Dietary Betaine Intake

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Tertile 1 (n=430)</th>
<th>Tertile 2 (n=431)</th>
<th>Tertile 3 (n=431)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAH, nmol/L</td>
<td>29.4±9.2</td>
<td>26.2±20.6</td>
<td>21.6±16.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SAM, nmol/L</td>
<td>91.3±11.6</td>
<td>93.4±13.1</td>
<td>95.7±15.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SAM/SAH ratio</td>
<td>4.49±2.87</td>
<td>5.58±3.70</td>
<td>6.46±4.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>15.4±5.88</td>
<td>14.7±5.16</td>
<td>13.5±4.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tCys, μmol/L</td>
<td>252.6±61.5</td>
<td>253.2±62.2</td>
<td>258.3±63.9</td>
<td>0.220</td>
</tr>
<tr>
<td>Folate, nmol/L</td>
<td>18.5±3.73</td>
<td>18.8±3.72</td>
<td>18.6±3.75</td>
<td>0.684</td>
</tr>
<tr>
<td>Vitamin B₁₂, pmol/L</td>
<td>294.5±112.0</td>
<td>288.1±106.4</td>
<td>301.4±104.1</td>
<td>0.260</td>
</tr>
</tbody>
</table>

1 Adjusted for age, sex, body mass index, smoking status, alcohol drinker, hypertension, diabetes mellitus, physical activity, family history of coronary artery disease, systolic blood pressure, gensini score, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, use or nonuse of statins, aspirin, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, and β-blockers, dietary energy intake, dietary protein intake, and dietary choline intake. SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; tHcy, total homocysteine; tCys, total cysteine.
Table 3. HRs for All-cause and Cardiovascular Mortality According to Dietary Betaine Intake in Patients With CAD

<table>
<thead>
<tr>
<th>Dietary Betaine Intake</th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
<th>P for Trend</th>
<th>Continuous Variable (1 SD Increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of deaths/person-years</td>
<td>110/3451</td>
<td>88/3618</td>
<td>61/3729</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted HR (95% CI)</td>
<td>1</td>
<td>0.77 (0.58, 1.02)</td>
<td>0.52 (0.38, 0.71)</td>
<td>&lt;0.001</td>
<td>0.75 (0.65, 0.85)</td>
</tr>
<tr>
<td>Age- and sex- adjusted HR (95% CI)</td>
<td>1</td>
<td>0.76 (0.57, 1.01)</td>
<td>0.52 (0.38, 0.72)</td>
<td>&lt;0.001</td>
<td>0.75 (0.66, 0.86)</td>
</tr>
<tr>
<td>Multivariable-adjusted Model 1 (95% CI) *</td>
<td>1</td>
<td>0.78 (0.59, 1.03)</td>
<td>0.61 (0.44, 0.83)</td>
<td>0.007</td>
<td>0.82 (0.72, 0.94)</td>
</tr>
<tr>
<td>Multivariable-adjusted Model 2 (95% CI) †</td>
<td>1</td>
<td>0.84 (0.63, 1.12)</td>
<td>0.72 (0.52, 0.99)</td>
<td>0.124</td>
<td>0.92 (0.80, 1.05)</td>
</tr>
<tr>
<td>Cardiovascular mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of deaths/person-years</td>
<td>80/3451</td>
<td>56/3618</td>
<td>31/3729</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted HR (95% CI)</td>
<td>1</td>
<td>0.67 (0.48, 0.95)</td>
<td>0.36 (0.24, 0.55)</td>
<td>&lt;0.001</td>
<td>0.62 (0.53, 0.74)</td>
</tr>
<tr>
<td>Age- and sex- adjusted HR (95% CI)</td>
<td>1</td>
<td>0.67 (0.47, 0.94)</td>
<td>0.37 (0.24, 0.56)</td>
<td>&lt;0.001</td>
<td>0.63 (0.53, 0.74)</td>
</tr>
<tr>
<td>Multivariable-adjusted Model 1 (95% CI) *</td>
<td>1</td>
<td>0.68 (0.48, 0.96)</td>
<td>0.44 (0.29, 0.66)</td>
<td>&lt;0.001</td>
<td>0.70 (0.58, 0.82)</td>
</tr>
<tr>
<td>Multivariable-adjusted Model 2 (95% CI) †</td>
<td>1</td>
<td>0.77 (0.54, 1.10)</td>
<td>0.55 (0.36, 0.84)</td>
<td>0.021</td>
<td>0.82 (0.69, 0.97)</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, body mass index, smoking status, alcohol drinker, hypertension, diabetes mellitus, physical activity, family history of coronary artery disease, systolic blood pressure, gensini score, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, use or nonuse of statins, aspirin, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, β-blockers, dietary energy intake, dietary protein intake, and dietary choline intake.

† Adjusted for variables in Model 1 plus S-adenosylhomocysteine, S-adenosylmethionine, total cysteine; total homocysteine, folate, vitamin B-12

HRs and 95% CIs were estimated by Cox proportional hazards regression models. CAD, coronary artery disease;
Figure 1. The relationships between dietary betaine intake and plasma concentrations of methionine metabolites (A) SAH, (B) SAM, (C) tHcy, and (D) tCys in patients with coronary artery disease. SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; tHcy, total homocysteine; tCys, total cysteine.
Figure 2. Kaplan-Meier plots for all-cause mortality (A) and cardiovascular mortality (B) according to tertiles of dietary betaine intake among patients with coronary artery disease. The cutoff points of tertiles of dietary betaine intake were 212 and 257 mg/d. $P$ values were compared by using the log-rank test.
Figure 3. Multivariable-adjusted spline functions for model 1 show the relationship between dietary betaine intake and all-cause mortality (A) and cardiovascular mortality (B) and Multivariable-adjusted spline functions for model 2 show the relationship between dietary betaine intake and all-cause mortality (C) and cardiovascular mortality (D). HRs and 95% CIs were estimated by Cox proportional hazards regression models (n=1292). Model 1 was adjusted for age, sex, body mass index, smoking status, alcohol drinker, hypertension, diabetes mellitus, physical activity, family history of coronary artery disease, systolic blood pressure, gensini score, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, use or nonuse of statins, aspirin, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, β-blockers, dietary energy intake, dietary protein intake, and dietary choline intake. Model 2 was adjusted for variables in Model 1 plus S-adenosylhomocysteine, S-adenosylmethionine, total cysteine; total homocysteine, folate, vitamin B-12.