Low serum betaine levels as a potential biomarker for type 2 diabetes mellitus in patients with coronary artery disease

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Abstract

Background

Dietary betaine intake was reported to associate with improved metabolic profile in type 2 diabetes (T2D). However, the role of circulating betaine in coronary artery disease (CAD) patients with T2D is still unknown. This study aimed to investigate the potential role of serum betaine as a potential biomarker for T2D risk in CAD patients.

Methods

Total 307 subjects were enrolled with 165 CAD patients (57 with T2D and 108 without) and 142 age and sex matched controls (CON). Fasting serum betaine were detected using liquid chromatography tandem mass spectrometry.

Results

Serum betaine were lower in simple CAD patients compared with healthy controls, and were further decreased in CAD patients with T2D. Betaine was inversely associated with fasting glucose. Subjects in the highest betaine tertile group had lower triglycerides, total cholesterol and low-density lipoprotein as well as lower percentage of CAD and T2D. Increased betaine in CAD was independently associated with low risk of T2D. Furthermore, betaine was a potential diagnostic marker in distinguishing simple CAD from CAD with T2D.

Conclusion

Low levels of betaine are associated with increased risk of CAD and T2D in CAD, and could be a biomarker for predicting T2D in CAD.

Background

Coronary artery disease (CAD) is a complex multifactorial disease which characterized by coronary artery stenosis and insufficient blood supply. Globally, CAD is the leading cause of death and morbidity, with approximately 17 million deaths per year [1–3]. Diabetes mellitus (DM), especial type 2 DM (T2D) is known to be an additional risk of CAD. The insulin resistance and lipid metabolism disorders in T2D patients were reported to promote pro-atherosclerotic effects and mechanistically involved in atherosclerosis [4–6]. The overall estimated number of adults with T2D in China has reached 100 million according to recent data, and about 63% remain undiagnosed due to unawareness [7]. More aggressive and diffuse development of atherosclerosis was found in CAD patients with diabetes, consequently, CAD patients with diabetes were shown to have worse 3-year functional outcome after treatment of CAD compared with simple CAD patients [8, 9]. Prevention strategies based on traditional risk factors such as obesity, hypertension and hyperlipidemia could not redeem the growing prevalence of CAD and T2D. Given this unfulfilled need for deeper understanding of these diseases, uncovering risk factors and

biomarkers based on metabolic profile of CAD and T2D for better understanding and risk prediction of T2D in CAD is of great importance.

Betaine is a nutrient of many important physiological functions such as methyl group donor and osmoprotectant. Betaine can be synthesized by oxidizing choline in the mitochondria in liver and kidney [10]. Besides the synthesis *in vivo*, dietary intake plays a critical role in the betaine content of the body [11]. Betaine involved in one-carbon metabolism are essential for the methylation and synthesis of DNA [12]. The change of betaine levels may indicate disorder in nutrient and physiological status [13].

Several studies have indicated the association of dietary betaine with cardiovascular disease (CVD) risk [14, 15]. Not only that, low betaine concentrations have been reported in subjects with metabolic syndrome [16]. In animal models, betaine treatment was also suggested to be inversely associated with inflammation which played a critical role in inducing metabolic disorder [17, 18]. However, less attention has been paid to the possible associations between circulating betaine and CAD patients with T2D. Thus, the present study explored the relationship between serum betaine levels and CAD patients with or without T2D by utilizing a hospital-based cross-sectional study among southern Chinese cohort, aimed to test whether the serum betaine could be used as a biomarker of CAD and CAD with T2D.

Materials And Methods

Study population and design

We randomly collected a total of 1395 blood samples from patients who were hospitalized for coronary angiography in Xiangya hospital at Central South University from June 2014 to September 2019. 165 CAD patients (CAD), aged 25–75, were included in this study. CAD was diagnosed as $\geq 50\%$ stenosis in at least one main coronary artery (determined by quantitative coronary angiogram analysis). Age and sex-matched control group (CON, $n = 142$) was an independently recruited set who underwent coronary artery CT or coronary angiography for chest pain but showed negative results. All participants were informed and provided written consent. We further divided CAD cases into CAD without T2D group (CAD-T2D, $n = 108$) and CAD with T2D group (CAD + T2D, $n = 57$). T2D was diagnosed according to standard world health organization criteria with fasting glucose ≥ 7.0 mmol/l or non-fasting glucose ≥ 11.1 mmol/l [19]. Subjects with an active infection, malignancy, severe liver or heart cerebrovascular diseases, severe proteinuria (> 3.5 g/day), intestinal dysfunction, gastrointestinal surgery history, choline or betaine supplementation in recent 6 months were excluded. Clinical information including age, sex, weight and body mass index (BMI) was retrospectively collected from each subject's medical records. Fasting blood samples were collected and immediately frozen at -80°C until analysis.

Laboratory Test

Betaine in serum were determined by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS) using D9-betaine internal standards as described previously [20]. Fasting blood

glucose (Glu), creatinine (Cr), triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), glutamic-pyruvic transaminase (AST), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured using HITACHI7170S automatic biochemical analyzer. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured by A&D TM-2655 automatic blood pressure measurement device.

Statistical analysis

Continuous data are presented as means $\pm$ standard deviations (SD) for normal distribution parameters or medians (interquartile ranges, IQR) for non-normal distribution parameters. Categorical variables are presented as numbers and percentages. Student's t-test or the Mann-Whitney U test was used for differences evaluation between two groups. Comparison between the tertiles was performed using one-way ANOVA. The Spearman correlation analysis was used to examine the associations between betaine and clinical variables. The association of betaine with T2D in CAD patients was evaluated by logistic regression analysis, adjustments were made for variables including: age, gender, BMI, Glu, Cr, TG and LDL. The concentrations of betaine were log-transformed before logistic analysis. The area under the receiver operating characteristics (ROC) curve was calculated to test the accuracy of betaine at discriminating CAD with T2D from CAD without T2D. Best cut-off value was calculated using the maximum sum of sensitivity and specificity. A two-tailed p value < 0.05 was considered statistically significant.

Results

The general clinical characteristics of participants with or without CAD are shown in Table 1. Mean age of CON, CAD-T2D and CAD + T2D groups were similar (57 ± 1 vs 59 ± 1 vs 58 ± 1 , $p = 0.2$). 40% of CON, 39% of CAD-T2D and 47% CAD + T2D were male ($p = 0.69$). We found no significant differences of height, weight, BMI, blood pressure, HR, TC, HDL and ALT levels between different groups ($p > 0.05$). Elevated Glu was observed in CAD + T2D ($7.13(6.15-9.08)$ mmol/l) group compared with CON ($5.1(4.67-5.63)$ mmol/l) and CAD-T2D groups ($5.02(4.72-5.81)$ mmol/l) ($p < 0.01$). CAD patients with or without T2D exhibited higher Cr, TG, AST and LDL levels compared with CON group ($p < 0.05$). Serum betaine concentrations were lower in CAD-T2D patients than CON ($28.29(22.38-35.73)$ μM vs $29.75(25.32-39.15)$ μM , $p = 0.02$) (Fig. 1). Remarkably, the betaine concentrations were even lower in CAD + T2D patients than CAD patients and CON ($24.14(20.84-30.76)$ μM vs $28.29(22.38-35.73)$ μM vs $29.75(25.32-39.15)$ μM , $p < 0.01$).

Table 1
Baseline characteristics of subjects stratified by disease status

	CON (n = 142)	CAD-T2D (n = 108)	CAD + T2D (n = 57)	p
Age (years)	57 ± 1	59 ± 1	58 ± 1	0.2
Male (n, %)	57 (40%)	42 (39%)	26 (47%)	0.69
Height (cm)	160(156–167)	160(155–168)	160(158–169)	0.8
Weight (kg)	62(55–72)	62(55–70)	68(59–75)	0.08
BMI (kg/m ²)	24(22–27)	25(22–26)	25(23–27)	0.16
SBP (mmHg)	126(116–144)	130(114–146)	133(120–150)	0.1
DBP (mmHg)	77(70–80)	80(69–89)	80(69–86)	0.33
HR	69(64–79)	72(66–80)	70(63–80)	0.94
Glu (mmol/l)	5.1(4.67–5.63)	5.02(4.72–5.81)	7.13(6.15–9.08)	< 0.01
Cr (μmol/l)	79(70.23–94.03)	85.3(73.7–98)	83.45(73.55–98.03)	0.03
TG (mmol/l)	1.33(0.98–1.84)	1.77(1.11–2.34)	1.88(1.34–2.55)	< 0.01
TC (mmol/l)	4.23(3.66–4.92)	4.55(3.89–5.54)	4.56(3.63–5.55)	0.11
HDL (mmol/l)	1.12(0.93–1.36)	1.11(0.91–1.32)	1.06(0.86–1.22)	0.06
LDL (mmol/l)	2.57(2.08–3.1)	2.9(2.25–3.59)	2.81(2.26–3.54)	0.01
ALT (U/l)	20.3(15.3–30.5)	25.2(15.8–39.7)	22.25(13.88–33.08)	0.17
AST (U/l)	21.95(18.6–28.33)	22.4(19.6–31)	20.21(16.28–27.25)	0.03
Betaine (μM)	29.75(25.32–39.15)	28.29(22.38–35.73)	24.14(20.84–30.76)	< 0.01
Continuous data are presented as mean ± SD or median (interquartile range), and categorical variables are presented as counts (%).				

Next, we categorized the total study cohort into tertiles according to distribution of serum betaine. As shown in Table 2, participants in tertile 1 with lowest betaine levels tend to have higher fasting glucose, TG, TC, LDL and ALT than those in tertile 2 and 3. Accordingly, we found the highest percentage of CAD and T2D in tertile 1, and the percentage of CAD and T2D further decreased significantly in tertile 2 and 3 with increased betaine concentrations ($p < 0.01$). No significant difference of other clinical characteristics such as age, gender, height, weight, BMI, blood pressure, HR, Cr, HDL and AST was observed between different tertiles. We then demonstrated an inverse correlation of betaine with fasting glucose by Spearman correlation analysis with $R = -0.17$ ($p < 0.01$) (Fig. 2).

Table 2
General characteristics of patients by tertiles of betaine levels

Tertiles of circulating betaine				
	Tertile 1 ($< 25.03 \mu\text{M}$) n = 103	Tertile 2 ($25.03\text{--}33.67 \mu\text{M}$) n = 102	Tertile 3 ($> 33.67 \mu\text{M}$) n = 102	p
Age (years)	57 ± 1	57 ± 1	58 ± 1	0.91
Male%	36%	46%	40%	0.33
Height (cm)	160(155–168)	162(158–167)	160(155–167)	0.67
Weight (kg)	65(57–75)	62(55.38–70)	62.5(55–69.75)	0.4
BMI (kg/m^2)	25(23–28)	24(22–26)	24(22–27)	0.4
SBP (mmHg)	130(116–146)	130(120–145)	128(113–141)	0.47
DBP (mmHg)	80(70–88)	77(70–84)	77(69–81)	0.11
HR	71(64–80)	69(64–79)	74(65–84)	0.2
Glu (mmol/l)	5.77(4.84–6.85)	5.2(4.75–6.06)	5.16(4.58–5.83)	< 0.01
Cr ($\mu\text{mol}/\text{l}$)	83.6(71.7–95.8)	83.2(73.6–97.3)	81.1(75.75–96.15)	0.57
TG (mmol/l)	1.92(1.3–2.79)	1.41(1.01–2.05)	1.36(1.04–1.85)	< 0.01
TC (mmol/l)	4.62(3.88–5.44)	4.27(3.62–5.29)	4.23(3.87–5.04)	0.03
HDL (mmol/l)	1.14(0.9–1.33)	1.06(0.92–1.26)	1.1(0.93–1.34)	0.15
LDL (mmol/l)	2.9(2.26–3.55)	2.65(2.08–3.19)	2.61(2.21–3.24)	0.05
ALT (U/l)	23.4(17.8–42.05)	22.5(14.9–32.9)	21.2(14.55–30.8)	< 0.01
AST (U/l)	21.2(18.25–31.8)	23.4(19.1–29.85)	22.5(19.05–27.55)	0.08
CAD%	69%	51%	41%	< 0.01
T2D%	30%	18%	8%	< 0.01
Continuous data are presented as mean $\pm$ SD or median (interquartile range), and categorical variables are presented as percentage.				

Based on logistic regression analysis, elevated betaine levels were independently associated with low risk of T2D in CAD patients unadjusted (OR = 0.05, 95% CI: 0.01–0.37, $p < 0.01$, model 1) or adjusted for traditional risk factors including age, gender, BMI, Cr, TG and LDL levels (OR = 0.04, 95% CI: 0.01–0.37, $p = 0.01$, model 2). When further adjusted for Glu, betaine remained to be a protective role in CAD with T2D but had no statistics significance (OR = 0.59, 95% CI: 0.03–13.46, $p = 0.74$, model 3) (Table. 3)

We performed receiver operating characteristic curve (ROC) analysis to explore the diagnostic role of betaine in diabetes. As shown in Figure. 3, the AUC was 0.62 and the best cut-off threshold of betaine was 24.63 μM in discriminating CAD with T2D from simple CAD (Fig. 3).

Discussion

This is the first study to demonstrate that circulating betaine levels are inversely associated with both CAD and the risk of type 2 diabetes in CAD. In the present study, subjects diagnosed with CAD had lower betaine levels than CON group (Table. 1). In addition, we observed further decreased betaine in CAD patients with T2D compared with simple CAD patients (Fig. 1). These results suggested that betaine levels might be used as a potential biomarker for CAD and CAD with diabetes mellitus, further studies are needed for validation on mechanisms in betaine related metabolic pathway.

Betaine was first identified in the plant as a nutrient in 19th century [11]. It can be synthesized from choline, which plays a critical role in lipid metabolism. Not only that, evidences from intestinal microbiology demonstrated that betaine is an important precursor of trimethylamine N-oxide (TMAO), a gut microbiota-dependent metabolite which is indicated as an independent prognostic risk factor for cardiovascular disease and metabolic syndrome [21–23].

However, contradictory results were reported with regard to betaine in cardiovascular disease. Higher dietary betaine intake was reported to be associated with a nonlinear higher risk of incident CHD and increased cardiometabolic mortality [24, 25]. Meanwhile, there's evidence that intake of betaine might increase cholesterol levels [26]. Results from Wang's study based on stable CAD participants suggested that plasma betaine levels were significantly higher in CAD cases than in healthy controls and there were dose-dependent associations with betaine concentration and the presence of CVD [27]. Nevertheless, a recent metabolomics study revealed opposite conclusions by showing no significant association between plasma betaine and incident atrial fibrillation in the population-based Framingham Study [28]. In the present study, we found reduced betaine levels in CAD cases compared with healthy controls (Table. 1). In addition, far less subjects in tertile 3 with highest betaine levels were diagnosed as having diabetes mellitus and CAD compared with tertile 1 and 2 which had relatively lower betaine in our study (Table. 2). Besides increased TG and TC, we also observed significantly higher ALT levels in low betaine tertiles, which were demonstrated to commonly elevate in acute coronary syndrome [29]. These results suggested a negative correlation of serum betaine levels with CAD risk. Notably, the median concentrations of betaine in CAD patients enrolled in Wang's study was higher than patients enrolled in this study (41.2 μM vs 26.83 μM). These differences may be caused by multiple characteristics between populations such as age structure, criteria of CAD patients, the Eastern and Western dietary habits and polymorphism of key metabolic enzyme. Further studies which involve all those factors are needed for more information.

T2D is a leading cause of microvascular complications and share many common effectors with CVD such as insulin resistance, hypertension and inflammation [30]. CVD patients with complication of diabetes have worse metabolic profile and increased mortality comparing with simple CVD patients [31].

Thus, elucidating the differences of onset mechanism between T2D and CAD, and identifying sensitive biomarkers for risk predicting of T2D in CAD is of great clinical importance for disease early diagnosis and prevention. Several studies have revealed the decreased betaine in patients with diabetes than those without [32, 33]. So far, no study has discussed the association of circulating betaine with risk of T2D in CAD. We, for the first time, compared betaine levels in CAD patients with or without T2D and revealed that CAD with T2D had remarkably lower betaine than simple CAD (Fig. 1). Results of the Spearman correlation analysis further confirmed the inverse association of serum betaine with fasting glucose (Fig. 2). These results indicated the association of decreased betaine with worse glucose metabolic profile and potential risk of diabetes. Betaine is a major methyl donor necessary to convert homocysteine into methionine by betaine:homocysteine methyltransferase (BHMT). Increased hepatic BHMT activity and mRNA levels were reported in diabetic rat model with a concomitant reduction in hepatic betaine concentration [34]. Besides, there were evidences indicated elevated urine excretion of betaine in diabetic patients [35]. These results might partly explain the decreased betaine in diabetes that may resulted in the accelerating transformation of betaine to dimethylglycine and excretion. However, more investigations involving expression and activity of BHMT and urine metabolic profile in CAD patients with or without T2D are warranted.

In addition, a study based on 4336 participants suggested that betaine levels were negatively correlated with total cholesterol and triglycerides [36]. We compared the clinical characteristics between different tertiles and revealed similar results that subjects in tertile 3 (highest in betaine) had lowest fasting glucose, triglycerides, total cholesterol and low-density lipoprotein compared with subjects in low betaine tertiles (Table. 2), which indicated an association of low betaine with deteriorated lipid profile and heightened risk of diabetes.

Li co-cultured betaine with high glucose induced mouse mesangial cells and found that betaine could inhibit cell proliferation and extracellular matrix deposition via regulating regulation of the Akt/Erk1/2/p38 MAPK signaling pathway [37]. Another data indicated that betaine could ease hepatic fat accumulation and promoted mitochondrial content and activity [38]. These evidences suggested that betaine was involved in the regulation of lipid and energy metabolism, which were key effectors in the pathogenesis of T2D and CAD. Another potential mechanism involved in the relationship of betaine with diabetes is inflammation. In vitro experiments demonstrated that betaine treatment could significantly inhibit NLRP3 inflammasome-related proteins and the levels of inflammatory cytokines including IL-1 β [39]. Therefore, high betaine levels may play a protective role in diabetes related metabolic disorders.

We further analyzed the association of betaine levels with T2D in CAD patients, logistic regression results suggested a protective role of betaine in T2D with very low odds ratio even after adjustments of multiple traditional risk factors (Table 3). These results demonstrated that serum betaine was independently associated with lower risk of T2D in CAD patients. Consequently, low betaine levels might serve as a biomarker for T2D risk in CAD. As confirmed in ROC analysis, betaine levels could partly predict the presence of T2D in CAD patients with an AUC of 0.62 (best cut-off = 24.63 μ M) (Fig. 3). Taken together, these results indicated the clinical utility of betaine for T2D risk prediction in CAD. Not only that, Grizales'

study discovered a reduced fasting glucose levels after orally supplement of betaine compared with placebo [40]. Long-term consumption of betaine has been shown to associate with decreased inflammatory factors [14]. Those studies suggested that betaine could be a potential therapeutic target in CAD and T2D as well. Further studies are needed to confirm this to be the case.

Table 3
Association of betaine levels with T2D in CAD patients

Model 1				Model 2			Model 3		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Betaine	0.05	0.01–0.37	< 0.01	0.04	0-0.37	0.01	0.59	0.03–13.46	0.74
Odds ratio shown were for plasma betaine in CAD patients with or without T2D. Model 1: unadjusted. Model 2: adjusted for age, gender, BMI, Cr, TG and LDL. Model 3: adjusted for all factors in model 2 plus Glu.									

Limitations

This study has several limitations. For example, the subjects in our study were enrolled in one center and the sample size was small. Therefore, selection bias cannot be excluded. And we did not include T2D patients without CAD as a control group, so we were unable to analyze the variations of betaine between T2D and CAD with T2D. Other potential confounding factors, such as nutritional status and activity of key metabolic enzyme, were not involved in this study, which might also influence the results.

Conclusions

The current study explored the correlation of serum betaine with CAD and CAD with T2D. CAD patients had reduced betaine levels than healthy controls. Not only that, CAD patients with even lower betaine were at more risk to complicate with T2D. Increased betaine levels were independently associated with lower risk of T2D in CAD patients, and betaine could be utilized as a potential biomarker for risk predicting and stratification of T2D in CAD.

Declarations

Acknowledgment

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All authors have no conflict of interest.

Ethical Approval and Consent to participate

All subjects were informed before enrolled in the study. The study plan was approved by the Ethical Committee of Xiangya Hospital of Central South University.

Consent for publication

The consent to publish was obtained from all participants in this study.

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Author contributions

D.O., Z.L., and F.G.: research idea, study design and writing manuscript. Y.Z., X.Q.: sample and data acquisition. F.G., Z.T.: sample analysis. F.G.: statistical analysis. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 2

Comparison of serum betaine levels in CON, CAD and CAD with T2D groups. CON, control group. CAD, CAD patients without type 2 diabetes. CAD+T2D, CAD patients with type 2 diabetes. Serum betaine levels were lower in CAD patients compared with healthy controls, and further decreased in CAD patients with T2D compared with those without.

Figure 4

Correlation analysis of serum betaine and fasting glucose. Glu, fasting glucose. Betaine was negatively correlated with Glu in CAD patients ($R = -0.17$, $p < 0.01$).

Figure 6

ROC curves of betaine for discriminating CAD from CAD with T2D. AUC indicates area under the receiver operating characteristic curve. For the best cut-off value of 24.63 µM, the likelihood of correctly predicting an event was up to 62% (AUC).