Journal of Diabetes and Its Complications 33 (2019) 107398

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Low serum choline and high serum betaine levels are associated with favorable components of metabolic syndrome in Newfoundland population

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ARTICLE INFO

Article history:
Received 22 April 2019
Received in revised form 26 May 2019
Accepted 11 June 2019
Available online 20 June 2019

Keywords:
Choline
Betaine
Metabolic syndrome
Newfoundland
Cross-sectional

ABSTRACT

Background: We investigated the relationships between serum choline and betaine levels with metabolic syndrome-related indices in the general population of Newfoundland.
Methods: 1081 adults were selected from the CODING study. Serum choline and betaine levels were measured. Major confounding factors were controlled in all analyses.
Results: Partial correlation and linear regression analysis showed that serum choline levels were positively associated with systolic blood pressure (r: 0.124), serum TG levels (r: 0.132) and negatively correlated with serum glucose levels (r: −0.121) in males ($p < 0.01$ for all). In females, serum choline levels were positively correlated with serum TG, TC and HDL levels (r: 0.104 to 0.148, $p < 0.05$ for all). Serum betaine levels were negatively associated with serum TG, TC, LDL and insulin levels, and with atherogenic index and HOMA-IR index in males (r: −0.081 to −0.179, $p < 0.05$ for all). In females, serum betaine levels were negatively associated with serum TG, hsCRP and insulin levels, and with HOMA-IR index (r: −0.092 to −0.213, $p < 0.05$ for all). Moreover, subjects with serum choline levels in the highest tertile showed highest serum TG levels and systolic blood pressure in males, and highest serum lipids levels in females. Subjects with the highest serum betaine levels had the lowest serum lipids levels, atherogenic index, IR severity in males, and the lowest serum TG and hsCRP levels, and IR severity in females.
Conclusion: Low serum choline and high serum betaine levels are associated with favorable components of metabolic syndrome in general adults.

1. Introduction

Choline and betaine are metabolically related quaternary ammonium compounds that are present in a number of different foods.[1] Choline is abundant in red meat, eggs, seafood, soybean and milk, whereas the main food sources for betaine are beets, cereal, grains, shellfish and spinach.[1] Both choline and betaine are important nutrients for human development and health. Choline is recognized as an essential nutrient[2] and plays important roles in signal transduction, lipid transport, neurotransmitter synthesis and methyl-group metabolism.[3] Betaine is a metabolite of choline metabolism and serves as an osmolyte in cells. Betaine is also an important methyl group donor for many pathways, including methylation of homocysteine to form methionine.[4] Numerous studies indicate that choline and betaine are involved in the pathogenesis of several disease conditions, including obesity,[5] cardiovascular diseases (CVD),[6] fatty liver,[7] and cancers.[8,9]

Metabolic syndrome (MS) is defined by a constellation of risk factors for type 2 diabetes mellitus (T2DM) and CVD, including insulin resistance (IR), obesity, hypertension, dyslipidemia, hyperglycaemia and their genetic susceptibility.[10] Epidemiologic studies indicate that MS confers a 5-fold increase in the risk of T2DM and 2-fold increased risk for developing CVD over the next 5 to 10 years.[11] Currently, MS affects 10% to 40% of individuals throughout the world and has become a major public-health challenge worldwide.[12] Evidence for the beneficial effects of choline and betaine on MS is found from both animal and human studies. Obesity is a characteristic of and an important trigger for MS.[13] Reports have shown that either choline or betaine supplements can improve obesity in animals, including rodents, pigs and chickens.[14,15] IR is a critical feature and key player in the mechanism

Conflicts of interest: We declare that we have no conflicts of interest.

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https://doi.org/10.1016/j.jdiacomp.2019.06.003
1056-8727/

of MS onset.[16] Dietary betaine supplements improve IR in mice.[15,17] In humans, our previous studies have shown that higher dietary choline and betaine intakes were significantly correlated with better body composition and lower IR in the adult population of Newfoundland, Canada.[18,19] A study in middle aged and elderly Chinese subjects showed that higher serum levels of betaine were correlated with favorable profiles of body composition, while serum concentrations of choline were negatively associated with body weight and BMI.[7] Our most recent publication has documented that higher serum choline and betaine levels are associated with favorable body composition characteristics in males.[20] Reports on studies examining the correlations between choline or betaine levels and MS-related components are rare. To date, only one cross-sectional study has investigated the relationships between serum choline and betaine levels with components of MS. This cross sectional study, performed in a middle aged and elderly Norwegian population of both males and females, showed that plasma betaine levels were negatively correlated with obesity, serum triglyceride levels and blood pressure, while plasma choline levels exhibited a positive association with serum triglyceride and glucose levels, and with obesity.[21] It is noteworthy that this study involved middle aged and elderly subjects only, and the blood samples used for choline and betaine detection were from non-fasting individuals. This can cause considerable variation in measurements of serum metabolites.[22,23] Research examining a large variety of different adult age groups with stricter standardization of blood sample collection and measurement of anthropomorphic data, is needed to provide a clearer picture of the relationships between measured serum choline and betaine levels and components of MS in the general adult population.

To our knowledge, no previous study has examined the relationships between serum choline and betaine levels with components of MS in the general adult population. To fill this knowledge gap, the current cross-sectional study was designed to examine the correlations between serum choline and betaine levels with components of MS, including blood pressure, serum lipids and glucose levels, and IR-related indices, in the general adult population of Newfoundland, and with strict control of major confounding factors. Our findings provide new insights into the relationships between serum choline, betaine levels and human health.

2. Methods and materials

2.1. Study population

Participants were selected from the CODING (Complex Diseases in the Newfoundland population: Environment and Genetics) study.[24,25] Inclusion criteria of CODING study are: 1) no <19 years of age; 2) at least a 3rd generation Newfoundlander; 3) without serious metabolic, endocrine or cardiovascular diseases and 4) not pregnant at the time of study.[19,20]

A total of 3214 participants were originally recruited in the CODING study. Among them, 820 individuals with incomplete data were excluded. 1081 participants were randomly selected based on the identification numbers from the remaining 2394 individuals and had serum choline and betaine levels measured.

Ethical approval of the study was received from the Health Research Ethics Authority (HREA), Memorial University, St. John's, NL, Canada, with Project Identification Code #10.33 (latest date of approval: February 11, 2016). All participants provided written and informed consent.

2.2. Anthropometric and body composition measurements

After a 12-hour fast, anthropometrics and body composition were measured. Body weight, standing height, waist and hip circumference were measured according to our previous reports.[18] BMI (kg/m^2) and Waist-to-hip ratio (WHR) were calculated. Blood pressure was measured by Dinamap 845 XT equipment (Criticon) after 10 min of seated resting and the mean values of 3 measurements were used for further analyses. Measurement of total percent body fat (BF%) was performed on a Dual Energy X-Ray Absorptiometry system.[24]

2.3. Lifestyle and dietary assessment

Information regarding participants' lifestyles, including demographics (age, gender and family origin), alcohol consumption, smoking status, disease status, medicine use, menopausal status was collected through a self-administered screening questionnaire. Physical activity patterns were measured using a separate ARIC Baecke Questionnaire.[18]

Dietary intake of the participants was assessed by a 124 item semi-quantitative Willett Food Frequency Questionnaire (FFQ).[19] A NutriBase Clinical Nutrition Manager (version 8.2.0; CybersoftInc, Phoenix, AZ) software package was used to process daily intake for each food item consumed, and the daily intake of total calorie (kcal/day) for each subject was computed automatically.[25]

2.4. Biochemical measurements

After a 12 hour fasting period, venous blood samples of the participants were collected. Serum samples were prepared by centrifugation and stored at −80 °C. Serum levels of glucose, triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-c) were measured on an Lx20 analyzer using Synchron reagents. Low-density lipoprotein cholesterol (LDL-c) was calculated using the formula: LDL-c = TC-HDL-c-TG/2.2. The atherogenic index (AI) was calculated with the formula: AI = (TC − HDL-c)/HDL-c.[26]

Fasting insulin (FINS) was measured on an Immulite Immunoassay analyzer. Homeostasis model assessment (HOMA-IR and HOMA-β)[27] were used to determine IR and β cell function:

$$\mathrm{HOMA{-}IR} = (\text{Fasting Insulin }[\mathrm{mU/L}] \times \text{Fasting Glucose }[\mathrm{mmol/L}])/22.5$$

$$\mathrm{HOMA{-}\beta} = (20 \times \text{Fasting Insulin }[\mathrm{mU/L}])/(\text{Fasting Glucose }[\mathrm{mmol/L}] - 3.5)$$

The quantitative insulin-sensitivity check index (QUICKI), another insulin sensitivity index used for the assessment of insulin sensitivity was also calculated.[28] The mathematical equation is shown below[28]:

$$\mathrm{QUICKI} = 1/[\log \text{fasting insulin } (\mathrm{mU/L}) + \log(\text{fasting plasma glucose } (\mathrm{mmol/L}) \times 18.0182)]$$

High sensitivity C-reactive protein (hsCRP) was measured by nephelometry according to the manufacturer's protocol (Beckman Coulter).

2.5. Serum choline, betaine levels measurements

Levels of serum choline and betaine were determined by a Liquid chromatography coupled tandem mass spectrometry (LC/MS/MS) method. The detailed method was described in our previous publication.[19] Briefly, protein-free serum added with internal standards (d9-choline and d11-betaine) was injected into a normal-phase Atlantis HILIC Silica HPLC column (3 μM, 2.1 × 100 mm, Waters Corporation, Milford, MA). Choline and betaine were eluted in an isocratic solvent system consisting of 25% ammonium formate (15 mM, pH 3.0) and 75% acetonitrile and delivered to the tandem Micromass Ultima Triple-Quad mass spectrometer (Waters Corporation, Milford, MA). The m/z transitions detected were: choline 104 > 60, d9-choline 113 > 69, betaine 118 > 59, d11-betaine 129 > 68. Calibration curves were prepared and quality control samples were included in each analytical run.

2.6. Statistical analyses

All data are presented as means ± standard deviation (SD). To perform effective statistical analysis, serum glucose, insulin and TG levels, HOMA-IR index, HOMA-β index, and total caloric intake were log-transformed to normalize the data distributions. Differences in anthropometrics, serum measurements, total caloric intake and physical activity level between women and men were assessed with independent Student's *t*-test.

Potential confounding factors were studied by investigating the correlations between components of the MS and serum choline or betaine levels with Pearson correlation analysis. Statistical interaction between serum choline, betaine levels and gender on the main outcomes was tested by analysis of covariance (ANCOVA). Partial correlation analysis controlling for age, total caloric intake, physical activity level, BF%, medicine status, alcohol, smoking and menopausal (only for females) was used to evaluate the correlations of serum choline, betaine levels with MS related indexes, including blood pressure, serum lipids levels and IR related indexes within male and female groups. Stepwise multiple linear regression analysis was used to evaluate the contribution of serum choline and betaine levels to MS related components within male and female groups. MS related components were used as dependent variables and serum choline, betaine levels, age, total caloric intake, physical activity level, BF%, medicine status, alcohol, smoking and menopausal (only for females) were used as independent variables.

To further explore the relationships between serum choline and betaine levels with MS related components, subjects were divided into tertiles (low, medium, or high) based on serum choline or betaine levels. Variations in the components of the MS among the three groups of different serum choline or betaine levels were assessed using ANCOVA controlling for age, total caloric intake, physical activity level, BF%, medicine status, alcohol, smoking and menopausal status (only for females).

Statistical analyses were all performed by SPSS 20.0 (SPSS Inc., Chicago, IL). All of the statistical tests were two sided and statistical significance was considered as $p < 0.05$. Statistical powers were above 80% ($\alpha = 0.05$, two-sided) and the sample sizes were large enough for all the tests.

3. Results

3.1. Physical parameters and serum choline, betaine levels

Demographic, physical and serum characteristics of the participants are shown in Table 1. Male subjects were on average 2.7 years younger than females. Weight, height, BMI, WC, WHR, Systolic BP, Diastolic BP, physical activity level and total dietary caloric intake were significantly higher in males, compared with females ($p < 0.001$). Male participants also had higher fasting glucose levels, serum TG levels, atherogenic index, HOMA-IR index, and HOMA-β index compared to females but had lower serum HDL-c levels, hsCRP levels and QUICKI index ($p < 0.01$). Compared to females, male subjects had significantly lower serum choline levels and higher serum betaine levels ($p < 0.001$). No significant difference was evident in hip circumference, serum TC, LDL-c and insulin levels between genders.

Table 1
Characteristics of the participants by gender.

Variables	Male	Female	*p*
Number	536	545	
Age (year)	42.2 ± 13.3	44.9 + 11.3	0.000
Weight (kg)	88.2 ± 15.7	70.3 + 13.2	0.000
Height (cm)	176.51 ± 6.15	162.30 ± 5.81	0.000
BMI	28.27 ± 4.58	26.7 + 5.0	0.000
Waist circumference (cm)	99.45 ± 12.64	91.25 + 13.59	0.000
Hip circumference (cm)	101.28 ± 9.94	102.53 + 11.42	0.055
WHR	0.98 ± 0.06	0.89 ± 0.07	0.000
Systolic BP (mmHg)	132.66 ± 14.84	122.51 ± 16.14	0.000
Diastolic BP (mmHg)	84.12 ± 10.17	80.42 ± 10.97	0.000
Physical activity	8.37 ± 1.56	7.99 ± 1.49	0.000
Dietary caloric intake (kcal/day)	2201.63 ± 1021.79	1823.06 ± 787.67	0.000
Fasting glucose (mmol/L)	5.32 ± 0.67	5.1 ± 0.67	0.000
Serum TG (mmol/L)	1.48 ± 0.99	1.17 ± 0.68	0.000
Serum TC (mmol/L)	5.08 ± 1.08	5.16 ± 0.97	0.199
Serum HDL-c (mmol/L)	1.19 ± 0.28	1.52 ± 0.38	0.000
Serum LDL-c (mmol/L)	3.22 ± 0.91	3.11 ± 0.89	0.057
Atherogenic index	3.44 ± 1.23	2.56 ± 1.05	0.000
Serum hsCRP (mg/L)	2.79 ± 4.48	3.55 ± 4.05	0.007
Serum insulin (pmol/L)	72.84 ± 49.15	67.31 ± 41.26	0.142
HOMA-IR	2.51 ± 1.81	2.24 ± 1.61	0.006
HOMA-β	399.11 ± 350.99	331.39 ± 339.08	0.000
QUICKI	0.34 ± 0.03	0.35 ± 0.03	0.009
Serum choline (μmol/L)	13.16 ± 3.21	13.84 ± 2.97	0.000
Serum betaine (μmol/L)	37.59 ± 11.32	31.11 ± 11.76	0.000

All values are means ± SD. BMI, body mass index; WC, waist circumference; WHR, waist-to-hip rate; Systolic BP, Systolic blood pressure; Diastolic BP, Diastolic blood pressure; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, Low-density lipoprotein cholesterol. Significant differences between female and male group were evaluated based on independence sample Student's *t*-test. Statistical significance was set to $p < 0.05$.

3.2. Correlations between serum choline, betaine levels and MS related indexes by gender

The correlations between serum choline and betaine levels with MS related indexes in different gender groups after controlling for the confounding factors are shown in Table 2. In male subjects, serum choline level was positively associated with systolic BP ($r = 0.124$, $p < 0.01$), serum TG level ($r = 0.132$, $p < 0.01$) and negatively correlated with serum glucose level ($r = -0.121$, $p < 0.01$). In females, serum choline level was positively related with serum TG, TC and HDL levels (*r* range from 0.104 to 0.148, $p < 0.05$ for all).

Serum betaine level was negatively associated with serum TG, TC, LDL-c, insulin levels, atherogenic index, HOMA-IR index and HOMA-β index (*r* range from −0.081 to −0.179, $p < 0.05$ for all) and positively related with QUICKI index ($r = 0.083$, $p < 0.05$) in male subjects. While, in female subjects, serum betaine level was negatively correlated with serum TG, hsCRP, insulin levels and HOMA-IR index (*r* range from −0.092 to −0.213, $p < 0.05$) and positively associated with QUICKI index ($r = 0.082$, $p < 0.05$).

To further identify the correlations between serum choline and betaine levels with components of the MS, we performed linear regression analysis. The findings are presented in Table 3. After all the confounding factors were controlled, serum choline levels were positively correlated with Systolic BP, serum TG level ($p < 0.01$ for both) and negatively correlated with fasting glucose levels ($p < 0.01$) in males. In female subjects, serum choline levels were positively related with serum TG, TC and HDL-c levels ($p < 0.05$ for all). Serum betaine levels were negatively associated with serum TG, TC, LDL-c, insulin levels, atherogenic index, HOMA-IR index, HOMA-β index, and positively correlated with QUICKI index in males ($p < 0.05$ for all). While, in females, serum betaine levels were negatively associated with serum TG, insulin and hsCRP levels, HOMA-IR index and positively correlated with QUICKI index ($p < 0.05$ for all).

3.3. Comparison of MS-related indexes in different serum choline and betaine levels groups

Subjects were divided into tertiles (low, medium, or high) based on serum choline or betaine levels. As presented in Table 4, analysis by ANCOVA showed a significantly positive and dose-dependent relationship between serum choline levels and systolic BP and serum TG levels in male subjects ($p < 0.05$), after controlling for the confounding factors. Serum glucose levels showed a significantly inverse and dose-dependent association with serum choline levels in males ($p < 0.01$).

Table 2
Partial correlations between serum choline and betaine levels with metabolic syndrome related indices in females and males.

	Choline (μmol/L)		Betaine (μmol/L)	
	Male	Female	Male	Female
	r1 (*p*)	r2 (*p*)	r1 (*p*)	r2 (*p*)
Systolic BP (mmHg)	0.124 (0.005)	0.020(0.647)	0.020 (0.649)	−0.034 (0.432)
Diastolic BP (mmHg)	0.004 (0.919)	−0.017 (0.693)	−0.067 (0.127)	−0.026 (0.557)
Serum TG (mmol/L)	0.132 (0.002)	0.127 (0.003)	−0.165 (0.000)	−0.213 (0.000)
Serum TC (mmol/L)	0.027 (0.536)	0.104 (0.016)	−0.179 (0.000)	−0.005 (0.909)
Serum HDL-c (mmol/L)	−0.017 (0.702)	0.148 (0.001)	−0.009 (0.839)	0.021 (0.629)
Serum LDL-c (mmol/L)	−0.006 (0.897)	−0.002 (0.961)	−0.130 (0.003)	0.044 (0.313)
Atherogenic index	0.028 (0.521)	−0.052 (0.232)	−0.155 (0.000)	−0.008 (0.858)
Serum hsCRP (mg/L)	−0.034 (0.518)	−0.053 (0.220)	0.007 (0.886)	−0.212 (0.000)
Fasting glucose (mmol/L)	−0.121 (0.006)	0.029 (0.509)	−0.038 (0.390)	0.013 (0.766)
Serum insulin (pmol/L)	0.062 (0.158)	−0.012 (0.784)	−0.081 (0.043)	−0.104 (0.016)
HOMA-IR	0.035 (0.427)	−0.005 (0.911)	−0.086 (0.021)	−0.092 (0.034)
HOMA-β	−0.008 (0.853)	0.000 (0.997)	−0.093 (0.050)	−0.061 (0.162)
QUICKI	−0.024 (0.588)	0.006 (0.889)	0.083 (0.023)	0.082 (0.039)

r1: Partial correlation coefficient controlled for age, total calorie intake, physical activity, BF%, medicine status, alcohol, smoking; r2 Partial correlation coefficient controlled for age, total caloric intake, physical activity, BF%, medicine status, alcohol, smoking and menopausal; Systolic BP, systolic blood pressure; Diastolic BP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, Low-density lipoprotein cholesterol. Statistical significance was set to $p < 0.05$.

In females, serum choline levels showed a significant positive and dose-dependent correlation with serum TG, TC and HDL-C levels ($p < 0.05$ for all). For each case, a 1 μmol/L increase in serum choline level, corresponded to a mean differences of 0.51 mmHg for Systolic BP, 29.28 μmol/L for TG, −20.5 μmol/L for glucose in males; and 44.59 μmol/L for TG, 42.99 μmol/L for TC, 12.74 μmol/L for HDL-c in females. No significant associations were found between serum choline levels with IR-related index in either males or females.

Table 3
Linear regression analysis of serum choline and betaine levels with metabolic syndrome related indices.

	Male (n = 536)			Female (n = 545)		
	R^2	β*	*p*	R^2	β*	*p*
Serum choline (μmol/L)						
Systolic BP (mmHg)	0.055	0.127	0.004	0.074	0.022	0.608
Diastolic BP (mmHg)	0.107	0.004	0.932	0.038	−0.014	0.746
Serum TG (mmol/L)	0.161	0.124	0.003	0.118	0.126	0.003
Serum TC (mmol/L)	0.111	0.024	0.573	0.117	0.099	0.019
Serum HDL-c (mmol/L)	0.085	−0.018	0.677	0.107	0.139	0.001
Serum LDL-c (mmol/L)	0.090	−0.006	0.884	0.102	−0.001	0.985
Atherogenic index	0.154	0.025	0.546	0.092	−0.043	0.314
Serum hsCRP (mg/L)	0.134	0.001	0.987	0.276	−0.039	0.299
Fasting glucose (mmol/L)	0.201	−0.109	0.007	0.137	0.036	0.388
Serum insulin (pmol/L)	0.284	0.051	0.185	0.152	−0.001	0.988
HOMA-IR	0.301	0.028	0.462	0.161	0.007	0.869
HOMA-β	0.298	−0.008	0.839	0.164	0.011	0.794
QUICKI	0.298	−0.019	0.622	0.162	−0.004	0.922
Serum betaine (μmol/L)						
Systolic BP (mmHg)	0.040	0.013	0.765	0.078	−0.039	0.392
Diastolic BP (mmHg)	0.112	−0.070	0.097	0.038	−0.024	0.606
Serum TG (mmol/L)	0.171	−0.158	0.000	0.149	−0.228	0.000
Serum TC (mmol/L)	0.136	−0.164	0.000	0.110	−0.002	0.963
Serum HDL-c (mmol/L)	0.085	−0.003	0.945	0.090	0.028	0.544
Serum LDL-c (mmol/L)	0.103	−0.118	0.006	0.105	0.051	0.262
Atherogenic index	0.173	−0.142	0.001	0.092	−0.009	0.844
Serum hsCRP (mg/L)	0.134	0.011	0.830	0.309	−0.199	0.000
Fasting glucose (mmol/L)	0.191	−0.049	0.225	0.137	0.010	0.828
Serum insulin (pmol/L)	0.283	−0.095	0.033	0.162	−0.106	0.016
HOMA-IR	0.302	−0.101	0.017	0.168	−0.093	0.033
HOMA-β	0.301	−0.106	0.030	0.167	−0.063	0.151
QUICKI	0.299	0.98	0.027	0.168	0.089	0.048

Models were multiple linear regression with statistical significant associations at $p < 0.05$. Systolic BP, systolic blood pressure; Diastolic BP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol. All analyses were controlled for age, total caloric intake, physical activity, BF%, medicine status, alcohol, smoking and menopausal (for female only). β*: standardized coefficient. R^2: Adjusted R square.

As shown in Table 5, serum betaine levels were significantly and inversely associated with Diastolic BP, serum TG, TC and insulin levels, AS-risk factor index, HOMA-IR and HOMA-β index ($p < 0.05$ for all), and positively correlated with QUICKI index in males ($p < 0.05$), after controlling all confounding factors. While, in females, serum betaine levels were inversely associated with serum TG, hsCRP, and insulin levels and HOMA-IR index, and positively correlated with QUICKI index ($p < 0.05$ for all). For each 1 μmol/L increase in serum betaine levels, the mean differences were −0.13 mmHg for Diastolic BP, −17.88 μmol/L for TG, −17.88 μmol/L for TC, −0.76 pmol/L for insulin, −0.026 for atherogenic index, −0.0289 for HOMA-IR, −5.50 for HOMA-β and 3.92×10^{-4} for QUICKI index in males; and −5.12 μmol/L for TG, −58.74 μg/L for hsCRP, −0.30 pmol/L for insulin, −0.013 for HOMA-IR and 7.87×10^{-4} for QUICKI index in females.

4. Discussion

In this relatively large population based cross-sectional study of general aged adults, we investigated the relationships of serum choline and betaine levels with components of MS, including blood pressure, serum lipids, serum glucose, serum hsCRP and IR-related indices, after properly controlling for major confounding factors. Significant associations were identified in the present study. Serum betaine levels were inversely associated with components of MS. However, serum choline levels were positively associations with MS-related indices. Moreover we revealed that some associations were gender specific. Briefly, we found that serum choline levels positively correlated with systolic BP, serum TG levels and negatively correlated with fasting glucose levels in males; and positively correlated with serum TG, TC and HDL-c levels in females. For betaine, negative correlations were observed with serum TG, TC, LDL-c and insulin levels, atherogenic index, HOMA-IR index, HOMA-β index, and positive correlation with QUICKI index in male subjects. While, in females, serum betaine levels were negatively correlated with serum TG, hsCRP and insulin levels, HOMA-IR index, and positively correlated with QUICKI index.

Our study has several strengths. First of all, this study involves examination of a broad variety of MS-related components. MS is comprised of multiple risk factors for T2DM and CVD, including obesity, dyslipidemia, hyperglycaemia, hypertension, and IR. Obesity and IR are also pathogenic factors for the condition.[29] In previous studies, we showed significant and negative correlations between serum choline or betaine levels with obesity in males, but not in females.[20] In the present study, four categories of MS-related components (blood pressure, serum lipid levels, fasting glucose and IR) were systematically studied. We also considered inflammation by examining levels of hsCRP because MS is

Table 4
Comparison of metabolic syndrome related indices according to serum choline levels.

	Choline (μmol/L)	Low	Medium	High	p[1]
Male	Number	178	179	179	
	Choline (μmol/L)	9.85 ± 1.33 (5.01–11.55)	12.92 ± 0.77 (11.55–14.26)	16.68 ± 2.25 (14.27–26.32).	0.000
	Age (year)	40.20 ± 11.74	40.26 ± 13.47	46.05 ± 13.67	
	Caloric intake (kcal/day)	2228.82 ± 995.12	2262.01 ± 1074.44	2113.85 ± 996.57	
	Physical activity	8.33 ± 1.53	8.43 ± 1.48	8.37 ± 1.68	
	Systolic BP (mmHg)	131.86 ± 13.74	130.79 ± 13.67	135.31 ± 16.65	0.037
	Diastolic BP (mmHg)	84.46 ± 9.75	83.11 ± 10.04	84.75 ± 10.69	0.716
	Serum TG (mmol/L)	1.37 ± 0.85	1.48 ± 1.10	1.57 ± 1.01	0.028
	Serum TC (mmol/L)	5.00 ± 1.07	5.09 ± 1.07	5.16 ± 1.10	0.571
	Serum HDL-c (mmol/L)	1.19 ± 0.27	1.18 ± 0.29	1.21 ± 0.27	0.571
	Serum LDL-c (mmol/L)	3.19 ± 0.91	3.20 ± 0.89	3.26 ± 0.92	0.885
	Atherogenic index	3.37 ± 1.21	3.52 ± 1.32	3.42 ± 1.15	0.147
	Serum hsCRP (mg/L)	2.95 ± 4.12	3.03 ± 5.84	2.53 ± 2.12	0.609
	Fasting glucose (mmol/L)	5.39 ± 0.70	5.33 ± 0.55	5.25 ± 0.74	0.013
	Serum insulin (pmol/L)	71.05 ± 45.65	73.66 ± 53.89	73.96 ± 47.85	0.560
	HOMA-IR	2.46 ± 1.59	2.53 ± 2.08	2.55 ± 1.72	0.810
	HOMA-β	392.02 ± 293.81	400.01 ± 420.08	406.37 ± 329.58	0.652
	QUICKI	0.34 ± 0.03	0.35 ± 0.04	0.34 ± 0.03	0.978
	Choline (μmol/L)	Low	Medium	High	p[2]
Female	Number	181	182	182	
	Choline (μmol/L)	10.79 ± 1.30 (6.16–12.32)	13.58 ± 0.69 (12.33–14.75)	17.07 ± 1.97 (14.75–24.83)	0.000
	Age (year)	41.82 ± 10.94	45.67 ± 11.16	47.14 ± 11.22	
	Caloric intake (kcal/day)	1867.68 ± 751.17	1801.69 ± 841.46	1791.80 ± 775.50	
	Physical activity	8.15 ± 1.45	7.96 ± 1.53	7.88 ± 1.49	
	Systolic BP (mmHg)	121.23 ± 15.33	122.22 ± 16.27	123.87 ± 16.51	0.899
	Diastolic BP (mmHg)	80.34 ± 10.58	80.36 ± 11.69	80.40 ± 10.67	0.776
	Serum TG (mmol/L)	1.04 ± 0.63	1.13 ± 0.63	1.32 ± 0.75	0.012
	Serum TC (mmol/L)	5.07 ± 1.02	5.08 ± 0.84	5.34 ± 1.03	0.035
	Serum HDL-c (mmol/L)	1.47 ± 0.35	1.54 ± 0.40	1.55 ± 0.40	0.012
	Serum LDL-c (mmol/L)	3.08 ± 0.88	3.01 ± 0.73	3.18 ± 0.87	0.239
	Atherogenic index	2.63 ± 1.17	2.47 ± 0.91	2.61 ± 1.00	0.071
	Serum hsCRP (mg/L)	3.45 ± 3.49	3.18 ± 3.87	3.92 ± 4.62	0.511
	Fasting glucose (mmol/L)	4.97 ± 0.55	5.09 ± 0.75	5.10 ± 0.69	0.588
	Serum insulin (pmol/L)	65.76 ± 36.60	63.81 ± 41.23	72.24 ± 45.70	0.605
	HOMA-IR	2.14 ± 1.40	2.15 ± 1.70	2.43 ± 1.72	0.730
	HOMA-β	302.05 ± 283.43	326.12 ± 388.70	365.30 ± 339.52	0.952
	QUICKI	0.35 ± 0.03	0.35 ± 0.03	0.35 ± 0.03	0.810

All values are means ± SD. Systolic BP, systolic blood pressure; Diastolic BP, diastolic blood pressure; BMI, body mass index; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.
p[1] controlled for age, total caloric intake, physical activity, BF%, medicine status, alcohol, smoking.
p[2] controlled for age, total caloric intake, physical activity, BF%, medicine status, alcohol, smoking and menopausal.

considered a pro-inflammatory state.[30] Atherogenic index was calculated and used in analyses due to the relevance of MS to cardiovascular risk.[31] Secondly, this study involved the systematic control of major confounding factors. It is essential to identify major confounding factors and to properly control for them in order to obtain reliable results in a large population based study such as this. MS is a complex pathological condition with various factors involved in its development.[32] Age and gender are primary factors that affect the development of MS related profiles.[32,33] Total dietary caloric intake and physical activity level are critical factors in keeping balance with energy gain, and can potently influence hypertension,[34] insulin sensitivity,[35] glucose and lipid metabolism.[32,33] Obesity is another well recognized condition that can lead to an elevated risk for hypertension, IR, and elevated serum lipids and glucose levels.[36] Moreover, menopause, alcohol consumption, smoking and medication were taken into consideration. Menopause is an important factor related with changes in sexual hormones and the MS components in females.[37] Alcohol consumption, smoking status and medication use are potential covariates that can influence energy intake, hypertension and insulin sensitivity.[38,39] In the current study, all of these confounding factors have been properly adjusted.

The most notable finding of this study was that for several components of MS, the correlations with serum betaine levels, were opposite that for serum choline levels. In addition, some gender differences were also observed. Serum choline levels were significantly associated with an unfavorable MS profile mainly for serum lipid levels. Serum choline levels were positively associated with serum TG levels in males, and with serum TG, TC and HDL-c levels in females. These results are consistent with those of Konstantinova et al., who reported a positive correlation between serum choline levels and several components of MS, including HDL and TG levels in middle aged and elderly men and women.[24] Choline is important to lipid metabolism, especially for the export of TG from the liver. Experimental studies have demonstrated that during choline-deficiency there is a shunting of free fatty acids into metabolically innocuous TG stores within the liver which might lead to decreased serum TG levels.[40] Choline supplementation in humans increases serum TG levels, but does not affect serum TC concentrations.[41] The significant associations between higher serum levels of choline with TG and TC levels deserve further study. We also found that serum choline levels positively correlated with blood pressure, and negatively correlated with fasting glucose levels in males. A study in rats demonstrated that dietary choline treatment resulted in increased blood pressure.[42] Serum choline levels were also reported to be negatively related with the risk for T2DM, characterized by high serum glucose levels.[43] Moreover, although we identified a significant relationship between higher dietary choline intake and lower IR,[44] no association was found between serum choline levels and IR in this study. It is recognized that the fasting serum choline or betaine are metabolites most affected by *in vivo* metabolic processes, and essentially

Table 5
Comparison of metabolic syndrome related indices according to serum betaine levels.

	Betaine (μmol/L)	Low	Medium	High	p^1
Male	Number	178	179	179	
	Betaine (μmol/L)	26.72 ± 3.52 (14.97–31.36)	35.77 ± 2.55 (31.44–40.20)	50.21 ± 9.21 (40.22–83.32)	0.000
	Age (year)	41.63 ± 13.23	42.10 ± 12.70	42.88 ± 13.86	
	Caloric intake (kcal/day)	2174.02 ± 1173.27	2177.13 ± 907.86	2253.59 ± 969.99	
	Physical activity	8.24 ± 1.46	8.47 ± 1.62	8.41 ± 1.60	
	Systolic BP (mmHg)	133.21 ± 13.43	131.05 ± 14.25	133.74 ± 16.63	0.184
	Diastolic BP (mmHg)	85.73 ± 10.16	83.81 ± 9.72	82.78 ± 10.47	0.041
	Serum TG (mmol/L)	1.68 ± 1.03	1.49 ± 1.03	1.26 ± 0.87	0.000
	Serum TC (mmol/L)	5.31 ± 1.18	5.05 ± 1.08	4.89 ± 0.92	0.014
	Serum HDL-c (mmol/L)	1.16 ± 0.28	1.20 ± 0.28	1.22 ± 0.28	0.600
	Serum LDL-c (mmol/L)	3.38 ± 1.01	3.19 ± 0.88	3.08 ± 0.79	0.083
	Atherogenic index	3.76 ± 1.33	3.41 ± 1.23	3.14 ± 1.04	0.003
	Serum hsCRP (mg/L)	3.10 ± 3.37	2.81 ± 6.37	2.57 ± 3.10	0.344
	Fasting glucose (mmol/L)	5.35 ± 0.69	5.35 ± 0.65	5.27 ± 0.67	0.142
	Serum insulin (pmol/L)	84.14 ± 52.90	68.21 ± 49.57	66.35 ± 42.83	0.005
	HOMA-IR	2.93 ± 1.99	2.37 ± 1.87	2.25 ± 1.45	0.005
	HOMA-β	473.98 ± 411.94	379.76 ± 358.80	344.81 ± 252.97	0.009
	QUICKI	0.34 ± 0.03	0.35 ± 0.03	0.35 ± 0.03	0.011
	Betaine (μmol/L)	Low	Medium	High	p^2
Female	Number	181	182	182	
	Betaine (μmol/L)	18.70 ± 4.50 (7.12–25.10)	30.47 ± 2.95 (25.13–35.37)	44.10 ± 7.92 (35.38–78.90)	0.000
	Age (year)	39.21 ± 12.68	47.25 ± 9.29	48.16 ± 9.45	
	Caloric intake (kcal/day)	1959.20 ± 850.70	1757.35 ± 749.30	1744.62 ± 750.23	
	Physical activity	8.27 ± 1.65	7.78 ± 1.40	7.94 ± 1.38	
	Systolic BP (mmHg)	121.43 ± 16.84	123.36 ± 15.16	122.53 ± 16.16	0.619
	Diastolic BP (mmHg)	79.31 ± 10.93	81.63 ± 10.57	80.16 ± 11.33	0.239
	Serum TG (mmol/L)	1.21 ± 0.66	1.20 ± 0.72	1.08 ± 0.66	0.000
	Serum TC (mmol/L)	4.95 ± 0.82	5.41 ± 0.95	5.14 ± 1.07	0.055
	Serum HDL-c (mmol/L)	1.52 ± 0.37	1.56 ± 0.41	1.49 ± 0.37	0.285
	Serum LDL-c (mmol/L)	2.85 ± 0.71	3.25 ± 0.82	3.17 ± 0.91	0.060
	Atherogenic index	2.43 ± 0.93	2.67 ± 1.01	2.62 ± 1.14	0.540
	Serum hsCRP (mg/L)	4.38 ± 4.15	3.16 ± 3.47	3.00 ± 4.27	0.000
	Fasting glucose (mmol/L)	4.91 ± 0.60	5.17 ± 0.82	5.08 ± 0.53	0.132
	Serum insulin (pmol/L)	70.03 ± 43.15	69.45 ± 42.64	62.33 ± 38.09	0.047
	HOMA-IR	2.40 ± 1.58	2.24 ± 1.79	2.08 ± 1.45	0.049
	HOMA-β	309.07 ± 307.71	375.00 ± 411.85	309.41 ± 285.55	0.124
	QUICKI	0.34 ± 0.03	0.35 ± 0.03	0.36 ± 0.03	0.047

All values are means ± SD. Systolic BP, systolic blood pressure; Diastolic BP, diastolic blood pressure; BMI, body mass index; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.
p^1 controlled for age, total calorie intake, physical activity, BF%, medicine status, alcohol consumption, smoking status.
p^2 controlled for age, total calorie intake, physical activity, BF%, medicine status, alcohol consumption, smoking status and menopausal.

independent of their dietary intakes.[45] Konstantinova *et al* has demonstrated that the plasma choline levels were not significantly associated with any identified dietary patterns, whereas betaine levels were negatively associated with a Western dietary pattern in middle-aged and elderly people.[46] No significant correlation between serum choline or betaine levels with the amount of their dietary intakes was identified in our study (data not shown). Further interventional studies are needed to verify the associations between choline levels and IR.

In contrast, serum betaine levels were associated with a favorable MS profile, especially serum lipid levels and IR-related indices. Serum betaine levels were negatively correlated with serum TG, TC, LDL-c levels in males, and serum TG levels in females. These results are consistent with a previous report. A cross-sectional study performed by Konstantinova et al. in middle aged and elderly participants showed that serum betaine levels were inversely associated with serum TG levels.[21] Our previous study demonstrated a significant association between betaine and reduced body fat indicating a beneficial effect of betaine in regulating systemic lipid profiles.[18,20] Animal studies also indicate that betaine supplementation decreased lipid levels in rodents,[15] consistent with our findings. Moreover, our study indicated that serum betaine levels were negatively correlated with insulin levels and the IR index in both females and males. Our previous study showed a significant negative correlation between dietary betaine intake and IR,[19] but to this point no study had investigated the relationship between serum betaine levels and IR. The present study has filled this gap and represents the first to reveal the negative correlation between serum betaine and IR. We also found serum betaine levels were negatively correlated with the atherogenic index in males, indicating the potential beneficial effect of betaine on reducing CVD risk. Measurement of blood levels of hsCRP is used to detect acute inflammatory processes. Detopoulou et al. suggested that dietary betaine intake was negatively correlated with concentrations of inflammatory markers in healthy adults.[44] Our results are consistent with this and demonstrate a negative correlation between serum betaine levels with serum hsCRP levels in females.

The different gender specific correlations in our study might be associated with the differences in serum betaine and choline levels in males and females. In our study, serum levels of betaine in men were 20.8% higher than in women, while serum choline levels were 5.2% lower in men compared with women. These differences could partially explain why the unfavorable associations between serum choline levels with the MS components in men were less than that for women, and the favorable associations between serum betaine levels with the MS components were stronger in men. Moreover, women have higher prevalence of cardiovascular disease than men in western countries.[21] Gender differences in serum levels and urinary excretion of betaine in predicting metabolic disorders were also reported by Lever et al.[47] Further study regarding the mechanisms for the gender differences is needed.

The potential mechanisms for the relationships between serum choline, betaine levels and components of MS are unclear. However, several mechanisms may partly explain the significant correlations shown here. Choline is a substrate in phosphatidylcholine synthesis. The formation of phosphatidylcholine is important for lipoprotein assembly, and hence plays critical role in the export of TG from the liver. Low circulating choline levels also facilitate the shunting of free fatty acids into metabolically innocuous TG stores within the liver and, thereby, decrease serum TG levels.[42] Betaine could promote fatty acid β-oxidation by enhancing carnitine palmitoyl transferase I-mediated free fatty acid translocation into the mitochondria for oxidation[48]; and decrease the capacity for fatty acid and TG synthesis through inhibition of acetyl-CoA carboxylase and fatty acid synthase,[49] leading to decreased lipid synthesis. By other mechanisms, betaine treatment increases insulin receptor substrate 1 phosphorylation and improves the downstream insulin signaling pathways.[17] Betaine supplementation also improves kinases 1/2 and protein kinase B activation to enhance insulin sensitivity.[18] Furthermore, betaine may improve insulin sensitivity by inhibiting adipose tissue inflammation.[44,50]

A number of limitations in the current study are considered. First of all, this study made use of a cross-sectional design, and thus cannot establish cause and effect relationships, but only indicate the direction and magnitude of relationships between variables. Furthermore, longitudinal or direct interventional studies with reasonable sample size are warranted to elucidate the mechanistic relationships mediating the observations made here. Secondly, although multiple confounding factors were adjusted in this study, the impact of genetic or other unknown and poorly measured residual confounding factors could not be ruled out.

In conclusion, we provide evidence that serum choline levels are significantly associated with an unfavorable MS phenotype and mainly through variation in serum lipid levels. In contrast, serum betaine levels are associated with a more favorable MS phenotype, especially related to serum lipid levels and IR-related indices in a general adult population from Newfoundland. In addition, these significant associations were independent of age, total caloric intake, physical activity level, alcohol consumption, smoking status, medication use, and menopausal status. A gender difference was also observed which needs further investigations. Our findings reveal novel insights into the relationships between serum choline and betaine levels with human health.

Acknowledgments

We would like to thank all of the volunteers who participated in this present study. We declare that we have no conflicts of interest.

Authors' contribution

GS and XG conceived and designed the experiments. XG and ER performed the experiments. XG and YT analyzed the data. XG draft the paper which was reviewed and modified by ER, HZ, and GS. All authors have read and approved the final manuscript.

Funding

This project has been funded by a Canadian Institutes of Health Research (CIHR) [operating grant: MOP192552] to Guang Sun. Xiang Gao was supported by the Natural Science Foundation of Shandong Province [No.ZR2019PC014].

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