Divergent Associations of Plasma Choline and Betaine with Components of Metabolic Syndrome in Middle Age and Elderly Men and Women[1,2]

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Abstract

Choline is involved in the synthesis of phospholipids, including blood lipids, and is the immediate precursor of betaine, which serves as a methyl group donor in a reaction converting homocysteine to methionine. Several cardiovascular risk factors are associated with plasma homocysteine, whereas little is known about their relationship to choline and betaine. We examined the relation of plasma choline and betaine to smoking, physical activity, BMI, percent body fat, waist circumference, blood pressure, serum lipids, and glucose in a population-based study of 7074 men and women aged 47–49 and 71–74 y. Overall plasma concentrations (means $\pm$ SD) were 9.9 ± 2.3 μmol/L for choline and 39.5 ± 12.5 μmol/L for betaine. Choline and betaine were lower in women than in men and in younger subjects compared with older ($P < 0.0001$). Multivariate analyses showed that choline was positively associated with serum triglycerides, glucose, BMI, percent body fat, waist circumference ($P < 0.0001$ for all), and physical activity ($P < 0.05$) and inversely related to HDL cholesterol ($P < 0.05$) and smoking ($P < 0.0001$). Betaine was inversely associated with serum non-HDL cholesterol, triglycerides, BMI, percent body fat, waist circumference, systolic and diastolic blood pressure ($P < 0.0001$ for all), and smoking ($P < 0.05$) and positively associated with HDL cholesterol ($P < 0.01$) and physical activity ($P < 0.0001$). Thus, an unfavorable cardiovascular risk factor profile was associated with high choline and low betaine concentrations. Choline and betaine were associated in opposite directions with key components of metabolic syndrome, suggesting a disruption of mitochondrial choline dehydrogenase pathway. J. Nutr. 138: 914–920, 2008.

Introduction

Choline and betaine are quaternary ammonium compounds, which are obtained from food or synthesized de novo in tissues. Choline, the major source of methyl groups in the diet, is obtained from eggs, beef, pork, liver, soybean, and wheat germ, whereas foods with the highest content of betaine are wheat bran, wheat germ, and spinach (1,2).

Phosphatidylcholine (lecithin), the most abundant choline species, is formed endogenously from phosphatidylethanolamine by a S-adenosylmethionine-dependent methylation reaction catalyzed by phosphatidylethanolamine N-methyltransferase. This is an important source of choline relative to dietary intake, especially in premenopausal women (3). Choline has a variety of biological functions by serving as an epigenetic regulator of gene expression (4) and a precursor of lipoproteins, membrane phospholipids, and the neurotransmitter acetylcholine; it is therefore important for lipid metabolism, the integrity of cell membranes, and nerve function (1).

Betaine is formed in kidney and liver by choline oxidation catalyzed by the mitochondrial enzyme, choline dehydrogenase (1,5,6). Betaine has 2 functions in humans. It is an organic osmolyte that accumulates in a variety of cells, including renal medullary cells, under condition of hypertonicity (7). Additionally, it serves as a methyl donor in the betaine-homocysteine methyltransferase (BHMT)[7] reaction (8), which is responsible for the betaine-dependent remethylation of homocysteine to methionine. This explains why plasma concentration of total homocysteine (tHcy) is decreased by betaine supplementation (9) and is inversely related to plasma betaine (10,11).

Plasma tHcy is an established risk factor for cardiovascular disease (CVD) (12,13), and shows a positive relation to a variety of CVD risk factors (14,15). Recent studies have demonstrated

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[7] Abbreviations used: BHMT, betaine-homocysteine methyltransferase; CVD, cardiovascular disease; HUSK, Hordaland Health Study; tHcy, total homocysteine.

that plasma tHcy increases with the number of components of metabolic syndrome (16,17), which refers to the cluster of risk factors related to central obesity, including elevated blood glucose, dyslipidemia, and hypertension (18).

The striking relations of tHcy with CVD risk factors (14), but also the role of choline in lipid metabolism and the function of betaine as an osmolyte (7), motivated us to investigate the association of choline and betaine in plasma with CVD risk factors, including components of metabolic syndrome (18). We examined these relations in a community-based study of 7074 men and women. Because the population distributions of choline and betaine are not well characterized, these are also presented.

Materials and Methods

Subjects. The Hordaland Health Study (HUSK) was conducted from 1997 to 1999 as a collaboration between the National Health Screening Service (now the Norwegian Institute of Public Health), the University of Bergen, and local health services (14). Of the total sample of 9187 men and women born in 1925–27 and 1950–51 who were invited to participate in this substudy of HUSK, 7074 (77%) agreed to participate. The participants underwent a brief health examination and donated a nonfasting blood sample. Information on lifestyle was collected via self-administered questionnaires. The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All subjects gave their written consent to participate in the study. We excluded 29 persons with missing choline and betaine levels; thus, the final number of participants was 7045.

Health examination and analytic procedures. The health examination included measurements of height, weight, and waist circumference. Blood pressure was measured after 10 min of seated resting using Dinamap 845 XT equipment (Criticon) and the mean values of 3 measurements were used for analyses. Body composition, including percent body fat, was measured by dual energy X-ray densitometry (EXPERT-XL; Lunar) (19). The level of physical activity was defined as a combination of intensity and frequency: no, low, moderate, and high activity. We defined smoking status as current smokers and nonsmokers (including former smokers). Energy intake was calculated from a FFQ (20) and at the time of the blood draw, we recorded time since the last meal (from 0 to 9 h).

Blood samples used for the preparation of plasma were collected from nonfasting subjects into evacuated tubes containing EDTA, placed in a refrigerator (4–5°C) within 15–30 min, and centrifuged, usually within 1 h (maximum within 3 h). EDTA plasma was stored at −80°C. Blood samples used for the preparation of serum were collected into evacuated tubes containing sodium sulphite titration gel and centrifuged within 2 h. The serum tubes were transported to the department of Clinical Chemistry, Ullevål University Hospital, Oslo, where glucose, total cholesterol, HDL cholesterol, and triglycerides were determined using an enzymatic method with reagents from Boehringer Mannheim, as adapted to a Hitachi 911 analyzer. HDL cholesterol was measured by a direct, enzymatic inhibition method. Serum lipids were determined within 7 d after collection of the sample. Non-HDL cholesterol was calculated as the difference between total and HDL cholesterol. Plasma choline, betaine, tHcy, and creatinine were determined by a method based on normal-phase chromatography-tandem MS (21). The concentration of plasma folate was measured by a *Lactobacillus casei* microbiological assay (22).

Statistical analyses. Augmented convex hull plots (23) were used for graphical presentation of choline and betaine distributions. Differences in mean plasma choline and betaine (mean ± SD) in 4 sex-age groups (men and women 47–49 and 71–74 y) were assessed by independent 2-sample *t* tests. The association between choline and betaine was evaluated by Spearman correlation analysis adjusted for age and sex.

Multiple linear regression analyses adjusted for age and sex were used to assess the differences in plasma choline and betaine according to several predictor variables. Continuous predictor variables [energy

intake, creatinine, folate, tHcy, systolic and diastolic blood pressure, BMI (kg/m^2), percent body fat, waist circumference, blood lipids, and glucose] were categorized in quartiles. In stratified analysis, quartiles were defined separately for each of the 4 age-sex groups. The mean difference of choline and betaine (mean, 95% CI) per increasing quartile of the predictor variable was used as the main effect measure. We also adjusted all associations for smoking, energy intake, time since last meal, plasma creatinine, and folate due to their possible confounding effect. We estimated sex- and age-adjusted mean difference in choline and betaine between individuals in the 25th and 75th percentiles of the predictor variables (Fig. 2). All statistical analyses were performed using SAS for Windows v 9.1 (SAS Institute).

Results

Choline and betaine were slightly positively skewed in all 4 age-sex groups (**Table 1; Fig. 1**). Overall, the plasma concentrations of choline and betaine were 9.9 ± 2.3 μmol/L and 39.5 ± 12.5 μmol/L, respectively. Men had higher concentrations of choline and betaine than women and in both genders, older subjects had higher plasma choline and betaine than younger subjects.

Plasma choline and betaine were positively correlated; the Spearman correlation coefficient, *r*, was 0.37 (*P* < 0.0001) after adjustment for age and gender. Choline and betaine decreased with increasing time since the last meal before the blood draw; age- and sex-adjusted regression coefficients were, respectively, −0.18 (95% CI: −0.20; −0.15) and −0.82 (95% CI: −0.97; −0.66) μmol/L per hour (*P* < 0.0001).

We estimated the differences in plasma choline and betaine between the lowest and the highest quartiles or contrasting groups of the predictor variable for all participants combined, adjusted for age and sex (**Fig. 2**). Both choline and betaine were positively associated with physical activity and folate and inversely associated with smoking and tHcy. Plasma choline showed a positive relation with plasma creatinine, BMI, percent body fat, waist circumference, serum triglycerides, and glucose and an inverse association with HDL cholesterol. Plasma betaine was positively associated with HDL cholesterol and inversely associated with BMI, percent body fat, waist circumference, systolic and diastolic blood pressure, serum triglycerides and non-HDL cholesterol (Fig. 2). Essentially the same results were obtained after additional adjustments for energy intake and time since last meal.

We also calculated the mean differences in plasma choline and betaine per increasing quartile or contrasting group of predictor variables in the total study population and for each of the 4 sex and age groups. The estimates were adjusted for smoking, creatinine, folate, and energy intake (**Tables 2,3**). Findings were essentially in agreement with those presented in Figure 2. Further adjustment for time since last meal did not materially change the associations. The associations were similar in the 4 sex-age groups. The most notable exception was the positive relation of choline to BMI, percent body fat, and waist circumference, which were significant only among women but showed a similar trend among men (Table 2). Thus, choline and betaine had divergent associations with the cluster of risk factors that are part of metabolic syndrome, i.e. BMI, percent body fat, waist circumference, systolic and diastolic blood pressure, serum triglycerides, HDL cholesterol, non-HDL cholesterol, and glucose.

Discussion

Principal findings. In this large population-based study of women and men 47–49 and 71–74 y old, we investigated the relation between plasma concentrations of choline and betaine and several established CVD risk factors. Plasma choline

TABLE 1 Plasma choline and betaine concentrations in men and women aged 47–49 y and 71–74 y in the HUSK[1]

	n		1	2.5	5	10	25	50	75	90	95	97.5	99
								Percentiles					
Choline							$\mu mol/L$						
All	7045	9.9 ± 2.3	5.6	6.2	6.7	7.2	8.3	9.6	11.1	12.8	13.9	15.0	16.7
Men													
47–49 y	1657	9.9 ± 2.1*,‡	5.8	6.4	6.9	7.4	8.4	9.7	11.1	12.6	13.5	14.7	16.2
71–74 y	1466	11.0 ± 2.5*,‡	6.7	7.1	7.5	8.1	9.3	10.8	12.4	14.1	15.6	16.6	18.7
Women													
47–49 y	2062	9.0 ± 1.9*,‡	5.1	5.7	6.3	6.8	7.7	8.9	10.2	11.5	12.5	13.2	14.2
71–74 y	1860	9.9 ± 2.3*,‡	5.7	6.1	6.7	7.2	8.3	9.6	11.1	12.8	13.9	14.9	16.6
Betaine													
All	7045	39.5 ± 12.5	17.5	19.7	22.4	25.5	31.0	38.2	45.9	55.1	61.0	67.6	77.7
Men													
47–49 y	1657	44.1 ± 12.6*,§	23.9	26.3	28.3	31.0	35.9	42.5	49.8	58.2	64.8	72.1	85.7
71–74 y	1466	45.5 ± 12.5*,§	24.1	26.5	28.9	31.6	36.8	43.6	52.2	61.1	67.6	73.8	83.1
Women													
47–49 y	2062	33.7 ± 10.8*,†	14.7	16.9	18.7	21.7	26.6	32.5	39.7	46.3	51.0	56.2	65.3
71–74 y	1860	37.3 ± 10.7*,†	18.9	20.4	22.3	24.9	29.8	36.0	43.2	50.3	56.6	62.3	70.4

[1] Values are means ± SD or percentiles. *$P < 0.0001$ for difference in choline or betaine between genders within age group (independent 2-sample t test). ‡$P < 0.0001$ for difference in choline between age groups within sex (independent 2-sample t test). §$P < 0.01$ for difference in betaine between age groups in men (independent 2-sample t test). †$P < 0.0001$ for difference in betaine between age groups in women (independent 2-sample t test).

showed a positive relation to serum triglycerides, glucose, BMI, body fat, and waist circumference, whereas plasma betaine was inversely related to these factors in addition to non-HDL cholesterol, and systolic and diastolic blood pressure, and positively related to HDL cholesterol. Thus, the most notable

FIGURE 1 Augmented density plots (23) of plasma choline and betaine in men and women aged 47–49 and 71–74 y. Gray curves show individuals 71–74 y and black curves show individuals 47–49 y. Dashed grey and black curves indicate women and solid grey and black curves indicate men. The contours show bivariate densities for plasma choline and betaine in all 4 sex-age groups. Small ellipses represent bivariate 95% CI for the means in each sex and age group. Marginal distributions are depicted on the sides of the main plot.

finding reported here is that choline and betaine are both associated with the cluster of CVD risk factors defined as metabolic syndrome (18) but in the opposite direction; choline is associated with an unfavorable CVD risk profile and betaine is associated with a beneficial CVD risk profile.
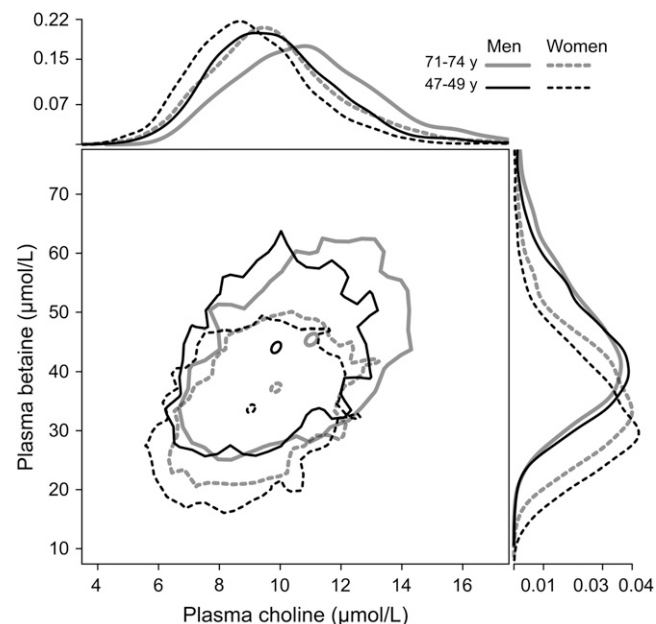
Strengths and weaknesses. Previous studies have found no association between CVD risk and intake of choline and betaine (24,25). To our knowledge, the present study is the first to examine the associations of blood concentrations with lifestyle and CVD risk factors. The study population is large and well-defined and ethnically homogenous.

One may argue that nonfasting blood samples with accompanying postprandial increases in blood glucose and triglycerides may attenuate the observed relations. However, recent studies suggest that postprandial triglyceride may be a stronger predictor of CVD risk than fasting levels (26). In addition, we determined non-HDL cholesterol, which is reliable in nonfasting serum and may be used for risk assessment instead of triglyceride and LDL levels, as was suggested by the Third Report of the National Cholesterol Educational Program Adult Treatment Panel III (27).

Because nonfasting samples were analyzed, it is possible that the choline and betaine concentrations reflected recent dietary intake. We found a moderate decline in both parameters as a function of time after last meal, but inclusion of this parameter in the regression model did not materially change the associations with CVD risk factors.

The collection of lifestyle data using self-reported questionnaires is a potential limitation, which could cause misclassification in the estimation of smoking habits and physical activity. Furthermore, we do not have information about the menopausal status of the youngest women. Finally, we measured only free choline and not other choline derivatives, such as phosphatidyl-choline, which is the most abundant choline-containing compound in human tissue (1).

Predictors of choline and betaine. In this study, plasma choline was higher in men than in women and in older than in
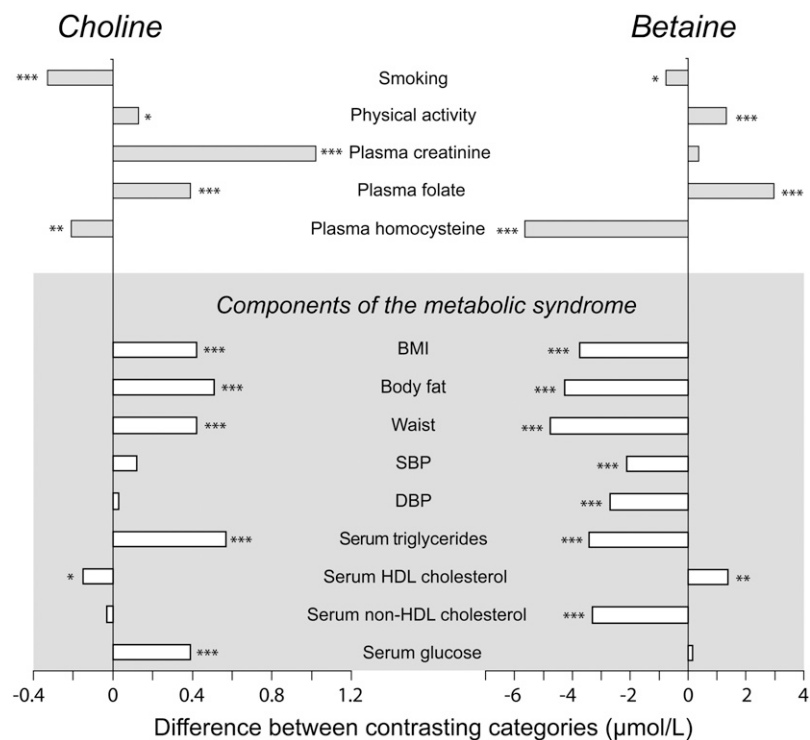
FIGURE 2 The relationship of plasma choline and betaine with major cardiovascular risk factors, including those associated with metabolic syndrome. The estimated age- and sex-adjusted differences in choline and betaine levels between contrasting groups (smoking and physical activity) or between individuals in the 25th and 75th percentiles of continuous predictor variables are shown. Two-sided P-value for the effect of predictor variable: ***$P < 0.0001$, **$P < 0.01$, *$P < 0.05$.

middle-aged participants. Choline showed a strong positive relation to betaine and a weaker positive relation to folate levels. These results are in agreement with published data on choline predictors (10,11,21). Our observation that choline is higher in

men than in women and in older women than in younger women are opposite to what may by expected from animal studies demonstrating stimulation of phosphatidylethanolamine N-methyltransferase and, thereby, phosphatidylcholine synthe-

TABLE 2 Difference in plasma choline per increasing quartile or contrasting group of metabolic syndrome components and other cardiovascular risk factors in men and women aged 47–49 y and 71–74 y in the HUSK[1,2]

Predictor	Total[3]	Men[4] 47–49 y	Men[4] 71–74 y	Women[4] 47–49 y	Women[4] 71–74 y
		μmol/L			
Age group[5]	−0.80 (− 0.92; −0.68)[a]				
Sex[6]	−0.63 (−0.77; −0.49)[a]				
Smoking[7]	−0.24 (−0.37; −0.11)[b]	−0.36 (−0.61; 0.11)[c]	−0.39 (−0.74; −0.04)[d]	−0.19 (−0.38; 0.005)	−0.20 (−0.51; 0.11)
Physical activity[8]	0.07 (−0.003; 0.15)	0.37 (0.14; 0.61)[c]	−0.07 (−0.34; 0.19)	−0.10 (−0.29; 0.08)	−0.12 (−0.38; 0.14)
Energy consumption	0.003 (−0.05; 0.06)	0.07 (−0.04; 0.17)	0.12 (0.001; 0.23)[d]	−0.06 (−0.14; 0.02)	0.0001 (−0.10; 0.10)
Plasma creatinine	0.32 (0.26; 0.37)[a]	0.13 (0.02; 0.23)[d]	0.47 (0.35; 0.59)[a]	0.11 (0.03; 0.19)[c]	0.42 (0.33; 0.52)[a]
Plasma folate	0.12 (0.07; 0.17)[a]	0.05 (−0.05; 0.16)	0.14 (0.02; 0.25)[d]	0.10 (0.02; 0.18)[d]	0.15 (0.05; 0.25)[c]
Plasma tHcy	−0.07 (−0.13; −0.02)[c]	−0.12 (−0.23; −0.01)[d]	−0.009 (−0.14; 0.12)	−0.12 (−0.21; −0.03)[c]	−0.04 (−0.15; 0.06)
BMI	0.10 (0.05; 0.15)[a]	0.05 (−0.06; 0.15)	0.04 (−0.08; 0.16)	0.16 (0.08; 0.24)[a]	0.13 (0.03; 0.23)[d]
Percent body fat	0.16 (0.08; 0.23)[a]	0.06 (−0.06; 0.18)	0.10 (−0.04; 0.24)	0.23 (0.15; 0.32)[a]	0.15 (0.03; 0.27)[d]
Waist circumference	0.12 (0.06; 0.17)[a]	0.01 (−0.09; 0.11)	0.06 (−0.06; 0.18)	0.14 (0.06; 0.22)[b]	0.10 (0.001; 0.20)[d]
Systolic blood pressure	0.02 (−0.04; 0.07)	0.03 (−0.08; 0.13)	0.02 (−0.09; 0.14)	−0.002 (−.08; 0.08)	0.12 (0.02; 0.21)[d]
Diastolic blood pressure	−0.01 (−0.06; 0.04)	0.02 (−0.08; 0.12)	−0.11 (−0.22; 0.006)	−0.08 (−0.16; 0.001)	0.08 (−0.02; 0.18)
Serum triglycerides	0.17 (0.12; 0.22)[a]	0.17 (0.07; 0.27)[b]	0.15 (0.04; 0.27)[c]	0.18 (0.10; 0.26)[a]	0.19 (0.09; 0.28)[b]
Serum HDL cholesterol	−0.06 (−0.12; −0.02)[d]	0.05 (−0.06; 0.15)	−0.06 (−0.18; 0.05)	−0.09 (−0.17; −0.006)[d]	−0.04 (−0.14; 0.05)
Serum non-HDL cholesterol	−0.01 (−0.06; 0.04)	0.02 (−0.08; 0.12)	−0.04 (−0.15; 0.08)	0.10 (0.02; 0.18)[d]	−0.03 (−0.12; 0.07)
Serum glucose	0.13 (0.08; 0.18)[a]	0.18 (0.08; 0.28)[b]	0.22 (0.10; 0.33)[b]	0.11 (0.03; 0.19)[c]	0.10 (−0.001; 0.19)
Plasma betaine	0.74 (0.69; 0.79)[a]	0.67 (0.58; 0.77)[a]	0.80 (0.69; 0.91)[a]	0.57 (0.49; 0.65)[a]	0.76 (0.66; 0.85)[a]

[1] Values are adjusted mean (95% CI) differences in plasma choline from multivariate linear regression models.
[2] Two-sided P-value for the effect of predictor variable. [a]$P < 0.0001$, [b]$P < 0.001$, [c]$P < 0.01$, [d]$P < 0.05$.
[3] Multiple linear regression analyses adjusted for sex, age group, smoking, plasma creatinine, folate, and energy intake.
[4] Multiple linear regression analyses adjusted for smoking, plasma creatinine, folate, and energy intake.
[5] 47–49 y vs. 71–74 y.
[6] Women vs. men.
[7] Smokers vs. nonsmokers (including former smokers).
[8] Moderate and high activity vs. no and low activity.

Predictor	Total[3]	Men[4] 47–49 y	Men[4] 71–74 y	Women[4] 47–49 y	Women[4] 71–74 y
		μmol/L			
Age group[5]	−2.82 (−3.45; −2.19)[a]				
Sex[6]	−9.02 (−9.77; −8.27)[a]				
Smoking[7]	−0.62 (−1.32; 0.08)	1.37 (−0.11; 2.86)	−2.52 (−4.34; −0.70)[c]	−0.79 (−1.84; 0.27)	−1.79 (−3.25; −0.32)[d]
Physical activity[8]	0.77 (0.36; 1.18)[b]	2.82 (1.42; 4.22)[a]	−0.37 (−1.74; 0.99)	1.20 (0.19; 2.20)[d]	0.42 (−0.80; 1.65)
Energy consumption	0.59 (0.30; 0.89)[a]	0.82 (0.20; 1.43)[c]	0.77 (0.17; 1.37)[d]	0.02 (−0.43; 0.46)	0.43 (−0.03; 0.88)
Plasma creatinine	0.03 (−0.28; 0.34)	0.29 (−0.34; 0.91)	−0.32 (−0.92; 0.29)[a]	−0.42 (−0.87; 0.03)	0.06 (−0.40; 0.51)
Plasma folate	0.99 (0.73; 1.26)[a]	0.48 (−0.14; 1.10)	1.44 (0.84; 2.04)[a]	0.23 (−0.22; 0.68)	1.72 (1.26; 2.18)[a]
Plasma tHcy	−1.79 (−2.09; −1.48)[a]	−1.48 (−2.12; −0.83)[a]	−2.00 (−2.66; −1.34)[a]	−1.63 (−2.12; −1.15)[a]	−1.76 (−2.27; −1.25)[a]
BMI	−1.30 (−1.57; −1.04)[a]	−2.10 (−2.71; −1.49)[a]	−1.53 (−2.14; −0.92)[a]	−0.81 (−1.26; −0.37)[b]	−1.45 (−1.92; −0.99)[a]
Percent body fat	−1.35 (−1.74; −0.96)[a]	−1.99 (−2.69; −1.28)[a]	−1.60 (−2.30; −0.91)[a]	−0.52 (−0.99; −0.06)[d]	−0.83 (−1.42; −0.24)[c]
Waist circumference	−1.55 (−1.85; −1.24)[a]	−2.11 (−2.71; −1.51)[a]	−1.43 (−2.03; −0.83)[a]	−0.77 (−1.22; −0.33)[b]	−1.45 (−1.90; −0.99)[a]
Systolic blood pressure	−0.73 (−1.03; −0.43)[a]	−1.17 (−1.79; −0.56)[b]	−1.06 (−1.65; −0.46)[b]	−0.62 (−1.07; −0.18)[c]	−0.04 (−0.50; 0.42)
Diastolic blood pressure	−0.86 (−1.13; −0.59)[a]	−1.30 (−1.91; −0.68)[a]	−1.28 (−1.87; −0.69)[a]	−0.89 (−1.33; −0.45)[a]	−0.27 (−0.73; 0.19)
Serum triglycerides	−1.07 (−1.34; −0.81)[a]	−0.87 (−1.48; −0.26)[c]	−1.49 (−2.09; −0.89)[a]	−1.28 (−1.73; −0.84)[a]	−0.97 (−1.42; −0.51)[a]
Serum HDL cholesterol	0.28 (−0.003; 0.56)	1.27 (0.65; 1.88)[a]	−0.08 (−0.63; 0.53)	0.16 (−0.30; 0.61)	0.30 (−0.16; 0.75)
Serum non-HDL cholesterol	−1.04 (−1.31; −0.77)[a]	−1.45 (−2.06; −0.84)[a]	−1.92 (−2.50; −1.32)[a]	−0.36 (−0.82; 0.09)	−0.80 (−1.26; −0.35)[b]
Serum glucose	0.05 (−0.22; 0.32)	0.03 (−0.58; 0.65)	0.14 (−.47; 0.74)	0.09 (−0.36; 0.54)	−0.25 (−0.71; 0.21)
Plasma choline	3.52 (3.27; 3.78)[a]	4.14 (3.56; 4.72)[a]	3.89 (3.32; 4.47)[a]	3.02 (2.60; 3.44)[a]	3.43 (3.00; 3.86)[a]

sis by estrogens (1,28), suggesting an additional effect from sex hormones on choline metabolism and distribution. The positive relation to folate is probably due to a choline-sparing effect of folate, which has been demonstrated in animal and human studies (29), whereas the positive association between choline and betaine is attributable to choline being the immediate metabolic precursor of betaine (8).

Compared with plasma choline, plasma betaine showed a similar but stronger relation to sex and folate in this and previous studies (8,10). The strong effect of gender has been explained by transcriptional regulation of human BHMT by estrogen and androgen (30), and the marked relation to folate by the function of betaine and 5-methyltetrahydrofolate as interchangeable sources of 1-carbon units (8,31).

Choline, betaine, and the cardiovascular risk factors. Some established CVD risk factors, including age, sex, smoking, physical activity, and tHcy levels (32), are associated with plasma choline and betaine. For these factors, the associations with elevated choline and betaine were essentially in the same direction, which could be expected for metabolites with a positive correlation and a close metabolic link.

Components of metabolic syndrome. The most notable finding from this study is that for several components of metabolic syndrome, associations with betaine and choline were in opposite directions. Because the correlation between choline and betaine is moderate, it is not inconsistent with divergent associations with a 3rd factor, i.e. key element(s) of metabolic syndrome.

Betaine is inversely associated with several components of metabolic syndrome (18) in the direction that decreases CVD risk. These factors include BMI, percent body fat, waist circumference, systolic and diastolic blood pressure, non-HDL cholesterol, HDL cholesterol, and triglycerides. These observations gain some support from results published by others. In subjects attending a lipid clinic, betaine was inversely related to body fat and Apo B (33). Betaine supplementation has been shown to reverse an atherogenic lipid profile in mice (34) and to decrease body fat in pigs (35,36). However, in humans, high doses of 4–6 g/d betaine increased total cholesterol, LDL cholesterol, and triglycerides (37,38). It is possible that endogenous and dietary betaine may have different effects on lipid metabolism and that the effects of betaine supplementation may vary according to dose, duration, and also between species.

Choline shows associations with BMI, body fat, waist circumference, HDL cholesterol, triglycerides, and blood glucose in the opposite direction (to betaine) but no association with non-HDL cholesterol and blood pressure. The relation between choline and blood lipids is actually in agreement with the observation that phosphatidylcholine supplementation in humans increases triglycerides but does not affect cholesterol concentrations (38). Experimental studies in mice show that high-fat feeding leads to weight gain, elevated triglycerides, hyperinsulinemia, and glucose intolerance in choline-replete animals, whereas choline-deficiency reduces glucose intolerance, possibly by shunting FFA into metabolically innocuous triglyceride stores in liver (39). Thus, betaine and choline are related to components of the metabolic syndrome, but in the opposite direction, despite their close metabolic connection and positive correlation.

Diet. Conceivably, the relations of plasma free choline and betaine to components of metabolic syndrome may reflect the influence of dietary patterns and recent intake of specific food items. We observed a weak inverse association between choline and betaine concentrations and time since last meal, but adjustment for this time interval did not materially affect the results. Furthermore, energy intake showed no relation to plasma choline but did have a positive relation to plasma betaine. Therefore, high energy intake does not explain the adverse associations of plasma choline and the strong inverse relation of plasma betaine to BMI, body fat, and waist circumference.

Metabolic syndrome has been linked to dietary intake. Although somewhat inconsistent results have been obtained, the prevailing view is that a healthy or prudent diet, with high amounts of fruits, vegetables, legumes, whole grain, poultry, and low-fat dairy products, is beneficial. An energy-dense diet rich in refined grains, cakes, sugar, red meat, fried food, and butter is adversely associated with metabolic syndrome (40–42). However, food items rich in choline (2) are not notorious components of an unhealthy dietary pattern. For example, in healthy adults, there was an inverse relation between choline intake and concentrations of inflammatory markers related to atherogenesis (43). Furthermore, high-fat dairy products, cakes, and sweets are low in choline. Likewise, the betaine content in most fruits and vegetable (except beets and spinach) is low (2).

Because intake of wheat bran and germ are major sources of betaine (2), it could be argued that the inverse association between plasma betaine and component of metabolic syndrome is attributable to the consumption of whole wheat. However, even though the interindividual variation of plasma betaine is substantial (up to 10-fold differences between individuals), the intraindividual variability is small, with an individual set point that remains stable for years (44). This suggests that plasma betaine is under strict metabolic control and justifies the concept of betaine status as a component of an individual's biochemical make-up.

Possible mechanisms. Central obesity and increased biologic activity of the upper visceral adipose tissue with excessive flux of fatty acids are regarded as primary factors of metabolic syndrome, leading to insulin resistance and atherogenic dyslipidemia (18). Both BHMT and choline dehydrogenase in rat liver are decreased by insulin and increased in diabetes (45). In addition, a general mitochondrial dysfunction prevails in metabolic syndrome (46), which may involve choline oxidation to betaine that takes place in the inner mitochondrial membrane (47). Thus, metabolic syndrome including insulin resistance may be associated with a disruption of the choline dehydrogenase pathway.

Recent studies suggest a role of BHMT in lipid metabolism. Dietary BHMT induction in rats resulted in increased liver ApoB mRNA, ApoB and triglycerides production, and VLDL secretion (48). BHMT is the most abundant protein in mammalian liver, is associated with other proteins, and binds to membranes. These are features suggesting roles in addition to homocysteine remethylation for this enzyme (30). Thus, BHMT may represent a metabolic link between lipid and 1-carbon metabolism.

This study demonstrates that key components of metabolic syndrome related to body composition, blood lipids, and glucose predict plasma choline and betaine; elevated choline was associated with a high CVD risk profile and elevated betaine was associated with a low CVD risk profile. We hypothesize that these divergent associations of the substrate (choline) and product (betaine) of mitochondrial choline dehydrogenase reflect disruption of this pathway under conditions of mitochondrial dysfunction in metabolic syndrome.

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