Dietary patterns, food groups, and nutrients as predictors of plasma choline and betaine in middle-aged and elderly men and women[1–3]

Svetlana V Konstantinova, Grethe S Tell, Stein E Vollset, Arve Ulvik, Christian A Drevon, and Per M Ueland

ABSTRACT

Background: Choline and betaine are linked to phospholipid and one-carbon metabolism. Blood concentrations or dietary intake of these quaternary amines have been related to the risk of chronic diseases, including cardiovascular disease and the metabolic syndrome.

Objective: We aimed to determine dietary predictors of plasma choline and betaine among middle-aged and elderly subjects recruited from an area without folic acid fortification.

Design: This is a population-based study of 5812 men and women aged 47–49 and 71–74 y, within the Hordaland Health Study cohort. Plasma concentrations per increasing quartile of intake of foods, beverages, and nutrients were assessed by multiple linear regression analysis, and dietary patterns were assessed by factor analysis.

Results: Plasma choline was predicted by egg consumption (0.16 μmol/L; $P < 0.0001$) and cholesterol intake (0.16 μmol/L; $P < 0.0001$), and betaine was predicted by consumption of high-fiber bread (0.65 μmol/L; $P < 0.0001$); high-fat dairy products (−0.70 μmol/L; $P < 0.0001$); complex carbohydrates, fiber, folate, and thiamine (0.66-1.44 μmol/L; $P \leq 0.0002$ for all); and total energy (0.45 μmol/L; $P = 0.004$). Plasma choline was not significantly associated with any identified dietary patterns, whereas betaine was negatively associated with a Western dietary pattern with a high loading for meat, pizza, sugar, and fat ($P < 0.0001$).

Conclusion: In this population of middle-aged and elderly men and women, recruited from an area with relatively low folate intake, neither plasma choline nor betaine was positively associated with consumption of animal products, fruit, or vegetables, but each was positively associated with the intake of specific food items such as eggs (choline) and bread (betaine). *Am J Clin Nutr* 2008;88:1663–9.

INTRODUCTION

Choline and betaine are quaternary amines with a close metabolic link (1–3). Choline and its derivatives have several biologic functions by serving as components of structural lipoproteins, blood, and tissue lipids and as a precursor of the neurotransmitter acetylcholine (1). Betaine acts as an osmolyte and as a methyl group donor in the remethylation of homocysteine to methionine, a conversion also catalyzed by a folate-dependent reaction (2, 4).

Both choline and betaine can be obtained from the diet (5). Choline is mostly present in foods of animal origin, such as liver, milk, meat, and eggs, whereas betaine is found in foods of plant origin, such as wheat bread, spinach, and beetroot (5, 6). Dietary recommendations for choline have been defined (6). In addition, both choline and betaine may be formed endogenously. Betaine is produced by oxidation of choline (2, 4), whereas the most abundant choline derivative, phosphatidylcholine, is synthesized from phosphatidylethanolamine, which is catalyzed by phosphatidylethanolamine methyltransferase (2).

Dietary intake of betaine and choline has recently been inversely associated with inflammatory markers related to atherosclerosis (7) and with plasma concentrations of total homocysteine (8). Supplementation with either choline (9) or betaine (10–12) decreases plasma total homocysteine. However, betaine has caused dyslipidemia in some studies (13, 14), but the alleged adverse effect on the lipid profile has been questioned (15). No relation has been found between betaine and choline consumption and the risk of cardiovascular disease (16).

Studies have shown that the concentrations of choline or betaine in serum or plasma are determined by a variety of biological and acquired factors, which include age, sex, the methylenetetrahydrofolate reductase (MTHFR) 677C→T polymorphism, smoking, exercise, folate status, and kidney function (17–20). Notably, several acquired factors that compose the metabolic syndrome (21), including triglycerides, body mass index (BMI; in kg/m^2), percentage body fat, and waist circumference, show divergent associations with plasma choline and betaine. An adverse cardiovascular risk factor profile is associated with high plasma concentrations of choline and low plasma concentrations of betaine (22).

Data on the effect of diet on betaine and free choline in serum or plasma are important to the consideration of whether the link to the metabolic syndrome reflects dietary pattern or a metabolic dysfunction (22). The Hordaland Health Study afforded the examination of plasma choline and betaine in relation to foods, beverages, nutrients, and dietary pattern in a large population of

[1] From the Department of Public Health and Primary Health Care (SVK, GST, and SEV) and the Section of Pharmacology, Institute of Medicine (AU and PMU), University of Bergen, Bergen, Norway; Haukeland University Hospital, Bergen, Norway (PMU); and the Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, Norway (CAD).

[2] Supported by the Western Norway Regional Health Authority (Project no. 911177), the Research Council of Norway, the Foundation to Promote Research into Functional Vitamin B-12 Deficiency, the Freia Chocolade Fabriks Medical Foundation, and the Johan Throne Holst Foundation for Nutrition Research.

[3] Reprints not available. Address correspondence to SV Konstantinova, Section for Epidemiology and Medical Statistics, Department of Public Health and Primary Health Care, University of Bergen, PO Box 7804, 5020 Bergen, Norway. E-mail: svetlana.konstantinova@isf.uib.no.

Received June 11, 2008. Accepted for publication September 5, 2008.
doi: 10.3945/ajcn.2008.26531.

TABLE 1
Descriptive characteristics of the study population in the Hordaland Health Study[1]

	Men		Women	
	47–49 y old (n = 1271)	71–74 y old (n = 1275)	47–49 y old (n = 1686)	71–74 y old (n = 1580)
Lifestyle				
Current smoker (%)	32[2]	16	34[2]	14
Low physical activity or none (%)	45[2,3]	57[3]	46[2]	77[4]
Vitamin supplement user (%)	19[3]	18[3]	32	31
BMI (in kg/m^2)	25.8[5] [26.1 (25.9, 26.2)][2,3,6]	25.8 [26.0 (25.8, 26.1)][3]	24.1 [24.8 (24.7, 25.0)][2]	25.8 [26.2 (26.0, 26.5)][4]
Plasma				
Choline (μmol/L)	9.66 [9.87 (9.75, 9.98)][2,3]	10.8 [11.0 (10.9, 11.2)][3]	8.89 [9.08 (8.98, 9.17)][2]	9.60 [9.83 (9.72, 9.95)][4]
Betaine (μmol/L)	42.3 [44.1 (43.4, 44.7)][2,3]	43.9 [45.6 (44.9, 46.3)][3]	32.7 [33.8 (33.3, 34.3)][2]	36.0 [37.2 (36.7, 37.7)][4]
Creatinine (μmol/L)	79.1 [79.5 (79.2, 80.7)][2,3]	83.7 [86.0 (85.2, 87.3)][3]	63.6 [64.4 (64.0, 65.0)][2]	66.8 [68.7 (68.1, 69.5)][4]
Food groups (g/d)				
Bread	215 [225 (220, 229)][2,3]	181 [189 (185, 193)][3]	157 [158 (156, 161)][2]	145 [145 (142, 148)][4]
Cereals	46.3 [53.9 (51.8, 55.9)][2,3]	17.0 [25.6 (24.2, 27.0)][3]	32.7 [39.5 (38.1, 40.8)][2]	12.4 [19.5 (18.5, 20.5)][4]
Cakes, pies, cookies	23.0 [29.0 (27.5, 30.4)][3]	22.0 [29.2 (27.6, 30.9)][3]	18.0 [24.1 (23.0, 25.2)]	15.0 [21.7 (20.5, 22.9)]
Vegetables	162 [199 (190, 208)][2,3]	154 [179 (172, 186)][3]	191 [226 (219, 233)][2]	153 [175 (169, 181)][4]
Fruit	204 [245 (235, 255)][2]	199 [232 (223, 241)]	220 [252 (245, 260)][2]	196 [233 (224, 241)]
Fish and seafood	83.9 [92.6 (89.3, 95.9)][2,3]	98.5 [106 (103, 109)][3]	65.4 [72.3 (70.2, 74.4)][2]	63.1 [70.7 (68.4, 73.0)][4]
Meat	138 [143 (139, 147)][2,3]	84.7 [90.4 (87.8, 93.0)][3]	98.3 [105 (102, 107)][2]	56.2 [61.4 (59.6, 63.3)][4]
Eggs	16.0 [18.0 (17.4, 18.7)][2,3]	15.6 [17.4 (16.7, 18.1)][3]	15.7 [15.6 (15.1, 16.0)][2]	15.3 [14.5 (14.0, 15.1)]
Milk	357 [391 (377, 406)][3]	303 [321 (310, 333)][3]	214 [255 (245, 264)]	284 [297 (287, 306)][4]
Sweets, sugar	11.0 [16.3 (15.3, 17.3)][2,3]	7.00 [11.7 (10.8, 12.6)][3]	9.00 [14.2 (13.3, 15.1)][2]	4.00 [8.68 (8.05, 9.30)]
Fats and oils	38.0 [42.5 (41.1, 43.9)][2,3]	29.5 [31.4 (30.4, 32.3)][3]	25.7 [29.0 (28.1, 29.9)][2]	17.7 [20.6 (19.9, 21.3)][4]
Beverages (ml/d)				
Coffee	420 [553 (533, 573)][2,3]	420 [399 (385, 414)][3]	420 [453 (439, 467)][2]	420 [356 (346, 367)][4]
Tea	0 [176 (158, 194)][3]	0 [186 (170, 203)][3]	200 [283 (264, 302)]	200 [235 (220, 250)][4]
Soft drinks, with sugar	90.0 [137 (126, 147)][2,3]	17.0 [70.1 (63.2, 77.0)][3]	35.0 [79.5 (73.6, 85.5)][2]	0 [47.8 (42.8, 52.7)][4]
Diet soft drinks	0 [64.3 (54.1, 74.5)][2]	0 [37.6 (30.3, 44.9)]	0 [63.7 (55.3, 72.3)][2]	0 [28.4 (23.3, 33.6)]
Water (tap and bottled)	270 [315 (296, 334)][2,3]	140 [239 (224, 254)][3]	300 [402 (383, 422)][2]	150 [292 (274, 311)]
Total alcoholic beverages	114 [171 (161, 182)][2,3]	32.0 [91.4 (82.0, 100.8)][3]	34.0 [64.9 (60.3, 69.6)][2]	0 [24.0 (21.3, 26.7)][4]
Beer	70.0 [132 (123, 142)][2,3]	4.00 [60.5 (52.4, 68.5)][3]	0 [28.1 (25.1, 31.0)][2]	0 [9.27 (7.60, 10.94)][4]
Wine	17.0 [32.8 (30.0, 35.6)][2,3]	0 [24.1 (20.7, 27.5)][3]	17.0 [34.9 (31.7, 38.1)][2]	0 [13.5 (11.8, 15.3)][4]
Spirits	1.00 [6.13 (5.41, 6.84)][3]	0 [6.85 (5.99, 7.71)][3]	0 [1.96 (1.71, 2.21)]	0 [1.21 (0.92, 1.50)][4]
Nutrient intake[7]				
Total energy (kJ)	10 393 [10 498 (10 361, 10 636)][2,3]	8265 [8420 (8304, 8535)][3]	7653 [7833 (7741, 7925)][2]	6330 [6413 (6320, 6506)][4]
Protein (g/d)	94.4 [97.2 (95.8, 98.6)][2,3]	77.3 [79.2 (78.0, 80.4)][3]	73.0 [74.9 (73.9, 75.8)][2]	60.4 [61.7 (60.8, 62.7)][4]
Total fat (g/d)	88.2 [91.9 (90.3, 93.4)][2,3]	68.4 [70.8 (69.6, 72.1)][3]	65.1 [68.0 (67.0, 69.1)][2]	50.8 [52.4 (51.4, 53.4)][4]
Saturated fat (g/d)	33.9 [34.8 (34.2, 35.4)][2,3]	26.3 [27.7 (27.2, 28.3)][3]	25.4 [26.6 (26.2, 27.0)][2]	20.4 [21.2 (20.7, 21.6)][4]
Monounsaturated fat (g/d)	28.1 [29.4 (28.9, 29.9)][2,3]	21.3 [22.1 (21.7, 22.6)][3]	20.7 [21.5 (21.2, 21.9)][2]	15.7 [16.3 (16.0, 16.6)][4]
Polyunsaturated fat (g/d)	18.7 [20.6 (20.1, 21.0)][2,3]	14.4 [15.2 (14.8, 15.5)][3]	13.6 [14.6 (14.3, 14.9)][2]	9.82 [10.6 (10.3, 10.8)][4]
VLC n–3 FA (g/d)[1]	0.77 [1.09 (1.04, 1.15)][3]	0.94 [1.19 (1.14, 1.24)][3]	0.56 [0.79 (0.75, 0.82)]	0.55 [0.80 (0.76, 0.84)]
Complex carbohydrates (g/d)	254 [260 (256, 264)][2,3]	213 [217 (214, 220)][3]	195 [198 (196, 201)][2]	171 [172 (170, 175)][4]
Sugars (g/d)	38.9 [45.2 (43.5, 46.9)][2,3]	30.4 [35.1 (33.8, 36.5)][3]	27.0 [32.0 (30.9, 33.1)][2]	21.8 [26.1 (25.1, 27.0)][4]
Fiber (g/d)	26.1 [27.0 (26.5, 27.5)][2,3]	23.7 [24.3 (23.9, 24.7)][3]	22.6 [23.2 (22.8, 23.5)][2]	19.9 [20.3 (20.0, 20.7)]
Cholesterol (mg/d)	303 [320 (314, 327)][2,3]	276 [284 (279, 290)][3]	247 [254 (250, 258)][2]	210 [218 (214, 223)]
Folate (μg/d)	334 [356 (349, 363)][2,3]	286 [303 (297, 309)][3]	289 [313 (307, 319)][2]	247 [265 (260, 271)]
Vitamin B-12 (μg/d)	7.40 [8.22 (7.98, 8.46)][2,3]	6.90 [7.70 (7.48, 7.93)][3]	5.50 [6.00 (5.86, 6.16)][2]	4.90 [5.49 (5.33, 5.65)]
Vitamin B-6 (mg/d)	1.69 [1.80 (1.76, 1.84)][2,3]	1.44 [1.53 (1.49, 1.56)][3]	1.36 [1.49 (1.45, 1.52)][2]	1.13 [1.26 (1.23, 1.29)]
Riboflavin (mg/d)	1.97 [2.10 (2.06, 2.15)][2,3]	1.59 [1.72 (1.68, 1.75)][3]	1.55 [1.70 (1.66, 1.73)][2]	1.35 [1.49 (1.45, 1.53)][4]
Thiamine (mg/d)	1.76 [1.86 (1.83, 1.90)][2,3]	1.46 [1.56 (1.53, 1.59)][3]	1.40 [1.52 (1.49, 1.54)][2]	1.18 [1.30 (1.27, 1.33)][4]
Alcohol (g/d)	6.20 [8.90 (8.37, 9.43)][2,3]	2.00 [5.92 (5.36, 6.47)][3]	2.30 [4.33 (4.03, 4.65)][2]	0 [1.71 (1.50, 1.92)]

[1] VLC, very-long-chain; FA, fatty acids.
[2] Comparisons between age groups within sex group, $P < 0.01$ (independent-sample t test or chi-square test as appropriate.
[3] Comparisons between sexes within age group, $P < 0.01$ (independent-sample t test or chi-square test as appropriate).
[4] Sex ×age group interaction for smoking, physical activity and vitamin supplement use (logistic regression analysis) and for other variables (linear regression analysis), $P < 0.01$.
[5] Median (all such values).
[6] $\bar{x}$; 95% CI in parentheses (all such values).
[7] Not including vitamin supplements.

men and women aged 47–49 and 71–74 y in Norway, where food is not fortified with B vitamins.

SUBJECTS AND METHODS

Subjects

The Hordaland Health Study was conducted from 1997 to 1999 as a collaboration among the National Health Screening Service (now the Norwegian Institute of Public Health), the University of Bergen (Norway), and local health services. Of the total sample of 9187 men and women born in 1925–27 and 1950–51 who were invited to participate in the Hordaland Health Study, 7074 (77%) participated. Participants underwent a brief health examination and donated a nonfasting blood sample. Information on diet and lifestyle was collected via self-administered questionnaires. The plasma choline and betaine values were measured in 7045 subjects (99.6%), and 6112 subjects (87%) also completed a food-frequency questionnaire (FFQ).

We excluded participants with reported energy intake below the 2.5th percentile (2118 kJ for women 71–74 y old and 3893 kJ for women 47–49 y old; 3855 kJ for men 71–74 y old and 5529 kJ for men 47–49 y old) and above the 97.5th percentile (11 133 kJ for women 71–74 y old and 13 086 kJ for women 47–49 y old; 14 023 kJ for men 71–74 y old and 17 621 kJ for men 47–49 y old). Thus, 131 men and 169 women were excluded from the analyses, for a final cohort of 5812 participants. There were no significant differences in plasma choline or betaine between users and nonusers of vitamin supplements. Therefore, the 2 groups were combined for analyses.

All subjects gave written informed consent. The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate.

Food-frequency questionnaire

In the present study, we used a slightly modified version of a previously described 167-item FFQ (23). The modified FFQ

TABLE 2
Mean difference in plasma choline and betaine concentrations (μmol/L) per increasing quartile or group of food intake in the Hordaland Health Study[1]

Food groups	Choline Mean (95% CI)	Choline *P*	Betaine Mean (95% CI)	Betaine *P*
White bread[2]	0.05 (−0.03, 0.14)	0.20	−0.22 (−0.66, 0.21)	0.31
Medium-fiber bread[2]	0.007 (−0.06, 0.07)	0.83	−0.29 (−0.63, 0.05)	0.09
High-fiber bread	0.01 (−0.04, 0.06)	0.67	0.65 (0.40, 0.89)	<0.0001
Cereals	−0.01 (−0.07, 0.04)	0.63	0.09 (−0.22, 0.40)	0.58
Cakes, pies, cookies	−0.05 (−0.10, 0.008)	0.09	0.08 (−0.21, 0.36)	0.60
All fruit	−0.005 (−0.06, 0.05)	0.85	0.08 (−0.20, 0.36)	0.56
Citrus fruit	−0.02 (−0.10, 0.06)	0.60	−0.33 (−0.75, 0.09)	0.12
All vegetables	0.02 (−0.03, 0.08)	0.41	0.13 (−0.15, 0.41)	0.35
Dried legumes	−0.07 (−0.12, −0.02)	0.005	−0.20 (−0.46, 0.06)	0.14
Mushrooms[2]	0.01 (−0.06, 0.09)	0.72	−0.43 (−0.81, 0.04)	0.03
All meat	0.04 (−0.02, 0.10)	0.21	−0.37 (−0.71, −0.04)	0.03
Nonprocessed meat	0.03 (−0.03, 0.09)	0.29	−0.47 (−0.76, −0.17)	0.002
Processed meat	0.03 (−0.03, 0.09)	0.33	−0.22 (−0.53, 0.10)	0.18
All fish and seafood	−0.02 (−0.08, 0.03)	0.42	0.19 (−0.10, 0.49)	0.20
Herring, mackerel	−0.04 (−0.09, 0.008)	0.10	0.11 (−0.14, 0.36)	0.39
Shellfish	−0.008 (−0.06, 0.04)	0.75	−0.34 (−0.60, −0.08)	0.01
Eggs	0.16 (0.11, 0.21)	<0.0001	0.12 (−0.15, 0.39)	0.37
All milk and dairy food	−0.02 (−0.07, 0.04)	0.52	−0.47 (−0.75, −0.20)	0.0008
Whole milk	−0.04 (−0.09, 0.01)	0.12	−0.39 (−0.65, −0.13)	0.004
Skim milk[2]	0.07 (0.008, 0.14)	0.03	0.07 (−0.27, 0.42)	0.68
Cream, sour cream, ice cream	−0.04 (−0.09, 0.02)	0.19	−0.70 (−0.98, −0.43)	<0.0001
All fats and oils	0.02 (−0.04, 0.08)	0.56	−0.35 (−0.67, −0.04)	0.03
Butter, butter mixtures[2]	−0.04 (−0.11, 0.04)	0.36	−0.74 (−1.13, −0.34)	0.0002
Margarine	−0.05 (−0.10, 0.006)	0.08	−0.14 (−0.43, 0.14)	0.33
Oil	0.02 (−0.03, 0.07)	0.42	0.09 (−0.18, 0.36)	0.51
Mayonnaise	0.003 (−0.05, 0.05)	0.89	−0.29 (−0.56, −0.02)	0.04
All sweets, sugar	−0.07 (−0.12, −0.02)	0.01	−0.58 (−0.87, −0.30)	<0.0001
Chocolate	−0.06 (−0.11, −0.01)	0.01	−0.54 (−0.80, −0.27)	<0.0001
Beverages				
Coffee	−0.10 (−0.15, −0.04)	0.0006	−0.10 (−0.40, 0.19)	0.48
Tea[2]	0.05 (−0.02, 0.11)	0.15	0.58 (0.23, 0.92)	0.001
Soft drinks, with sugar	−0.03 (−0.08, 0.02)	0.20	−0.44 (−0.71, −0.17)	0.001
Total alcoholic beverages	0.02 (−0.03, 0.08)	0.33	−0.46 (−0.75, −0.17)	0.002
Beer[2]	0.09 (0.008, 0.17)	0.03	0.04 (−0.37, 0.45)	0.84
Wine	−0.02 (−0.07, 0.03)	0.37	−0.60 (−0.86, −0.33)	<0.0001
Spirits[2]	−0.0002 (−0.07, 0.07)	0.99	−1.06 (−1.44, −0.67)	<0.0001

[1] Values are mean (and 95% CI) differences in plasma choline and betaine per increasing quartile or group of predictor variable from multivariate linear regression models adjusted for age group, sex, energy intake, and time since last meal. Two-sided *P* values are for the effect of food group or food item.

[2] Categorized in 3 groups: nonuse compared with 2 equal groups of increasing consumption.

includes frequency alternatives (from 1 time/mo to several times/d), the number of units eaten, and portion sizes (eg, slices, glasses, cups, pieces, or spoonfuls) to capture the habitual diet during the past year.

The information from the FFQ is presented as individual food or beverage items, food groups, and nutrients. Individual food items corresponded to the items listed on the questionnaire, whereas food groups included related food items (eg, the "bread" food group contained bread with low, medium, and high amounts of fiber).

In addition to individual food items, the FFQ included questions about the most commonly used brands of single-vitamin and multivitamin supplements on the market at the time of the study. Daily nutrient intakes were computed from a database and software system developed at the Department of Nutrition, University of Oslo (KBS software, version 3.2; University of Oslo, Oslo, Norway). The nutrient database is mainly based on the official Norwegian food composition table with an update on folate content from 2001 (24). For the dietary pattern analysis, we assigned each of the 167 single-food items in the questionnaire to 1 of 41 nonoverlapping food groups, so that each group contains foods with similar ingredients.

Health examination and analytic procedures

A brief examination included measurements of height and weight. Nonfasting blood samples used for the preparation of plasma were collected into evacuated tubes containing EDTA, placed in a refrigerator (at 4–5 °C) within 15–30 min, and then centrifuged, usually within 1 h (maximum: 3 h). Tubes containing EDTA-plasma were stored at −80 °C. Plasma free choline, betaine, total homocysteine, and creatinine concentrations were measured by using a high-throughput method based on normal-phase liquid chromatography–tandem mass spectrometry detection (18).

Statistical analysis

Differences in consumption of food groups and nutrients between age groups and sexes were assessed by using independent-sample *t* tests or chi-square tests. Sex × age group interactions were tested by logistic regression analysis or linear regression analysis. Associations of plasma choline and betaine with predictor variables (food groups and nutrients) were analyzed by multivariate linear regression. All regression analyses were conducted separately for women and men. Because the observed associations were similar for both sexes, results are presented for the total sample, adjusted for age group, sex, energy intake, and time since last meal.

Continuous predictor variables (ie, food groups, beverages, and nutrients) were categorized in quartiles, with the exception of refined and medium-fiber bread, mushrooms, skim milk, butter, tea, beer, and liquor. Because of low consumption of these foods and beverages (<50% of participants), 3 categories were used: nonusers and 2 equally large groups of users. We used 2 multivariate models: model 1 was adjusted for age group, sex, energy intake, and time since last meal, and model 2 was as model 1 with

TABLE 3
Mean difference in plasma choline and betaine concentrations (μmol/L) per increasing quartile of nutrient intake in the Hordaland Health Study[1]

Nutrient	Choline Mean (95% CI)	Choline *P*	Betaine Mean (95% CI)	Betaine *P*
Energy[2]	−0.03 (−0.09, 0.03)	0.30	0.45 (0.14, 0.75)	0.004
Protein	0.07 (−0.02, 0.16)	0.13	0.09 (−0.39, 0.57)	0.72
All fat	−0.007 (−0.10, 0.09)	0.88	−0.85 (−1.34, −0.36)	0.0007
Saturated fat	−0.03 (−0.12, 0.05)	0.42	−0.99 (−1.43, −0.55)	<0.0001
MUFA	0.04 (−0.05, 0.13)	0.42	−0.82 (−1.29, −0.35)	0.0007
PUFA	0.03 (−0.04, 0.11)	0.39	0.28 (−0.11, 0.67)	0.16
n–6 PUFA	0.04 (−0.04, 0.11)	0.34	0.10 (−0.28, 0.48)	0.61
n–3 PUFA	0.003 (−0.07, 0.08)	0.93	−0.06 (−0.44, 0.33)	0.78
VLC n–3 FA	−0.01 (−0.06, 0.04)	0.69	0.30 (0.02, 0.58)	0.03
Cholesterol	0.16 (0.09, 0.23)	<0.0001	−0.27 (−0.62, 0.08)	0.13
Complex carbohydrates	0.02 (−0.07, 0.10)	0.73	1.44 (0.98, 1.91)	<0.0001
Simple carbohydrates	−0.05 (−0.11, 0.01)	0.11	−0.30 (−0.60, 0.01)	0.06
Fiber	0.007 (−0.06, 0.07)	0.82	0.76 (0.43, 1.10)	<0.0001
Folate	0.03 (−0.03, 0.10)	0.33	0.66 (0.32, 1.01)	0.0002
Vitamin B-12	0.009 (−0.05, 0.07)	0.77	−0.03 (−0.36, 0.29)	0.85
Vitamin B-6	0.01 (−0.06, 0.08)	0.74	0.32 (−0.04, 0.68)	0.08
Riboflavin	−0.02 (−0.08, 0.05)	0.63	0.18 (−0.16, 0.52)	0.31
Thiamine	0.02 (−0.05, 0.09)	0.53	0.80 (0.42, 1.17)	<0.0001
Retinol equivalents	−0.01 (−0.07, 0.05)	0.72	0.08 (−0.21, 0.37)	0.60
β-Carotene	0.02 (−0.03, 0.07)	0.51	0.22 (−0.06, 0.49)	0.12
α-Tocopherol	−0.006 (−0.07, 0.05)	0.84	0.27 (0.04, 0.59)	0.08
Vitamin D	−0.02 (−0.08, 0.03)	0.38	0.22 (−0.06, 0.50)	0.13
Vitamin C	−0.005 (−0.06, 0.05)	0.86	−0.01 (−0.30, 0.27)	0.92
Alcohol	−0.004 (−0.06, 0.05)	0.88	−0.69 (−0.97, −0.41)	<0.0001

[1] MUFA, monounsaturated fat; PUFA, polyunsaturated fat; VLC, very-long-chain; FA, fatty acids. Values are mean (95% CI) differences in plasma choline and betaine per increasing quartile of predictor variable from multivariate linear regression models adjusted for age group, sex, energy intake and time since last meal. Two-sided *P* values are for the effect of nutrient.

[2] Not adjusted for energy.

The American Journal of Clinical Nutrition

additional adjustment for smoking, physical activity, dietary folate, and vitamin B-6. In each analysis, the main effect measure was the mean difference in choline or betaine per increasing quartile, or contrasting group, of the independent variable.

Major dietary patterns were obtained by principal component analysis. Three factors with eigenvalues >2, which together accounted for 21% of the total variation, were extracted on the basis of the scree test and evaluation of the loading matrix after orthogonal (varimax) rotation. The derived factors (food patterns) were labeled Western, Healthy, and Traditional. We categorized participants by quartiles of dietary pattern scores, and we calculated mean differences in plasma choline and betaine per increasing quartile of dietary pattern score by using multivariate linear regression analyses after adjustment for age group, sex, energy intake, and time since last meal. We also adjusted for smoking, BMI, and physical activity to control for possible confounding by these lifestyle factors.

All statistical analyses were performed by using SAS for WINDOWS software (version 9.1; SAS Institute Inc, Cary, NC).

RESULTS

Population characteristics and diet

Descriptive statistics and dietary habits of the study population are shown in **Table 1**. There were significant age and sex differences in the consumption of most food items, beverages, and nutrients. The highest consumption of bread, meat, and eggs was observed in middle-aged men, and the lowest consumption was observed in elderly women. A similar age distribution was observed for cholesterol and saturated fat. Consumption of vegetables was highest in middle-aged women. Consumption of fruit in men and women did not differ significantly.

Choline and betaine according to dietary items and beverages

The concentrations of plasma choline and betaine according to intake categories of food groups and beverages were measured by linear regression after adjustment for age, sex, energy intake, and time since last meal (**Table 2**). The highest mean differences in plasma choline concentrations per increasing quartile of food item were observed for eggs (0.16 μmol/L; $P < 0.0001$) and coffee (−0.10 μmol/L; $P \leq 0.0006$), whereas no significant association with meat consumption (processed and nonprocessed) was observed.

Plasma betaine showed the strongest positive association with high-fiber bread (0.65 μmol/L per quartile; $P < 0.0001$). The strongest inverse associations per increasing quartile of food group were seen for butter and cream (−0.74 and −0.70 μmol/L, respectively; $P \leq 0.0002$), sweets and chocolate (−0.58 and −0.54 μmol/L, respectively; $P < 0.0001$), nonprocessed meat (−0.47 μmol/L; $P = 0.002$), and alcoholic beverages (−0.46 μmol/L; $P = 0.002$). No significant associations were seen between plasma betaine and fruit and vegetables (except mushrooms). The associations listed in Table 2 were essentially the same after additional adjustment for smoking, physical activity, dietary folate, and vitamin B-6 (data not shown).

Choline and betaine according to nutrient intake

The concentrations of choline and betaine in plasma according to nutrients were assessed in a similar linear regression model (after adjustment for age group, sex, energy intake, and time since last meal (**Table 3**). Among the nutrients, only cholesterol showed a significant association with plasma choline (0.16 μmol/L per quartile; $P < 0.0001$), whereas plasma betaine was positively associated with several nutrients, including complex carbohydrates, fiber, folate, and thiamine (0.66–1.44 μmol/L per quartile; $P \leq 0.0002$). The strongest inverse associations with plasma betaine were seen for saturated fat, all fat, and monounsaturated fat (−0.99, −0.85 and −0.82 μmol/L, respectively; $P \leq 0.0007$) and for alcohol (−0.69 μmol/L per quartile; $P < 0.0001$). These associations were materially not changed after additional adjustments for smoking, physical activity, dietary folate, and vitamin B-6 (data not shown). Plasma betaine but not

TABLE 4
Food group loadings for 3 dietary patterns extracted by principal components analyses in the Hordaland Health Study[1]

Food groups	Dietary pattern		
	Western	Healthy	Traditional
Refined bread	—	—	—
Medium-fiber bread	0.31	−0.34	—
High-fiber bread	—	0.20	—
Pizza	0.58	—	—
Rice, pasta, flour	0.43	0.42	—
Breakfast cereals	—	—	—
Cakes	0.28	—	—
Potato	—	−0.22	0.61
Carrots	−0.23	0.20	0.54
Cabbage, kohlrabi	—	0.23	0.42
Cauliflower, broccoli	—	0.54	0.22
Onions, salads, spinach	—	0.52	—
Cucumber, tomato, pepper, lettuces	—	0.62	—
Mushrooms	—	0.50	—
Other fresh vegetables	—	0.58	—
Mixed, frozen, and preserved vegetables	—	0.44	—
Dried legumes	0.21	—	0.30
Fruits, berries	—	0.30	0.34
Juices	—	0.21	—
Whole red meat	0.55	—	0.38
Total processed meat	0.69	—	—
Liver	—	—	—
Chicken	0.33	0.29	—
Lean fish	—	—	0.65
Fatty fish	—	—	0.42
Fish products	0.27	—	0.45
Shellfish	0.27	—	—
Eggs	0.26	—	0.20
Milk	—	−0.21	0.21
Yogurt	—	0.21	—
Cream, sour cream, ice cream	0.30	—	—
Cheese	—	—	—
Margarine, butter	0.31	—	—
Oil	0.32	0.26	0.30
Mayonnaise, dressing	0.39	—	—
Sweets, sugar	0.34	—	—
Snacks	0.44	—	—
Coffee	0.34	−0.22	—
Tea	—	0.34	—
Sugared or diet soft drinks	0.39	—	—
Alcoholic beverages	0.41	—	—
Total variation explained (%)	10.1	6.2	5.1

[1] Values <0.20 were excluded for simplicity.

plasma choline was positively related to energy intake (0.45 μmol/L per quartile; $P = 0.004$).

Choline and betaine according to dietary patterns by factor analysis

We identified 3 dietary patterns by factor analysis (**Table 4**). The factors were labeled Western (ie, high in meat, refined grain products, fat, and sugar), Healthy (ie, high in vegetables and fruit), and Traditional [ie, high in potatoes, fish, and (cooked) vegetables]. In a regression model with adjustment for age group, sex, energy intake, and time since last meal, plasma choline and betaine were not positively associated with any of the identified dietary factors. Plasma betaine was inversely related to the Western pattern ($P < 0.0001$; **Table 5**). After additional adjustment for smoking, BMI, and physical activity, the association with the Western dietary pattern became slightly weaker but was still statistically significant ($P = 0.0001$).

DISCUSSION

Principal findings

In this study of 5812 men and women aged 47–49 or 71–74 y, egg consumption was the only dietary item that predicted plasma choline, whereas plasma betaine was most strongly associated with high-fiber bread intake and inversely related to high-fat dairy products, sugar-containing foods and beverages, and alcoholic beverages. Among nutrients, only cholesterol was associated with plasma choline, whereas betaine was associated with complex carbohydrates, fibers, folate, thiamine, and total energy intake. Choline and betaine were not positively associated with any of the identified dietary patterns.

Strengths and weaknesses

The present study is the first population-based study of dietary predictors of plasma choline and betaine. The large size of the study population provides precise estimates of differences in plasma choline and betaine concentrations in relation to dietary intake of food items and nutrients. We used a validated 167-item quantitative FFQ (23, 25). Folate, fruit, and vegetable intakes estimated by this FFQ in the present population were previously found to be highly correlated with plasma folate concentrations (26). Unfortunately, the Norwegian food-composition tables, and thus the nutrient database, do not include information on choline and betaine content, and the relation between intakes and plasma concentrations of choline and betaine was not assessed in the present study.

Information on lifestyle factors and time since last meal was also available, which allowed the investigation of dietary predictors after control for possible confounders, including the moderate postprandial increase in choline and betaine (18). A potential limitation is related to the collection of dietary data using an FFQ, which has inherent problems of inaccuracy and potential misclassification of dietary intake (27, 28). Nonfasting plasma samples were collected for practical reasons, and there could be residual confounding from recent food consumption even after adjustment for time since last meal.

We measured free choline, but food contains various choline species, including water-soluble (ie, choline, phosphocholine, and glycerophosphocholine) and lipid-soluble (ie, phosphatidylcholine and sphingomyelin) forms (1) in amounts that are correlated (5). Once absorbed, they are metabolized and are interconvertible, but the relation and flux between different choline pools are complex and not fully understood.

Dietary items and nutrients

Our observation that plasma choline showed the strongest associations with egg consumption and cholesterol intake is in accordance with the high content of phosphatidylcholine (5) and cholesterol (29) in eggs. Other dietary sources of choline, such as meat and other animal products (5), were not related to plasma choline concentrations. This observation is consistent with the results from the factor analyses, where the dietary pattern denoted Western did not predict plasma choline.

The strongest predictors of plasma betaine were high-fiber bread and nutrients such as complex carbohydrates, fiber, folate, and thiamine. These relations may be explained by the high betaine content of grain products (5), which are also rich in complex carbohydrates, fiber, folate, and thiamine (30). In addition, folate has a sparing effect on betaine, as suggested by the increase in plasma betaine after folic acid supplementation (20).

Dietary pattern and the metabolic syndrome

We previously reported that plasma choline and betaine show divergent association with several components of the metabolic syndrome. Choline showed a positive relation to serum triglycerides, glucose, BMI, body fat, and waist circumference, whereas

TABLE 5
Food patterns as determinants of choline and betaine in the Hordaland Health Study[1]

	Dietary pattern					
	Western		Healthy		Traditional	
	Mean (95% CI)	P^2	Mean (95% CI)	P^2	Mean (95% CI)	P^2
Choline						
Model 1[3]	−0.004 (−0.08, 0.07)	0.91	−0.0008 (−0.06, 0.05)	0.98	0.04 (−0.02, 0.11)	0.15
Model 2[4]	−0.002 (−0.08, 0.07)	0.96	−0.03 (−0.08, 0.03)	0.34	0.03 (−0.03, 0.09)	0.27
Betaine						
Model 1[3]	−0.98 (−1.38, −0.58)	<0.0001	0.02 (−0.27, 0.31)	0.90	0.11 (−0.21, 0.42)	0.52
Model 2[4]	−0.78 (−1.18, −0.38)	0.0001	−0.04 (−0.34, 0.25)	0.77	0.24 (−0.07, 0.56)	0.13

[1] Values are mean (95% CI) differences in plasma choline and betaine per increasing quartile of dietary score from multivariate linear regression models.
[2] Two-sided P values are for the effect of dietary score.
[3] Adjusted for age group, sex, energy intake, and time since last meal.
[4] Adjusted for age group, sex, energy intake, time since last meal, smoking, BMI, and physical activity.

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 (22).

Several studies have shown that a healthy diet characterized by high amounts of fruit, vegetables, legumes, whole grain, poultry, and low-fat dairy products is associated with lower risk of the adverse components of the metabolic syndrome, whereas an energy-dense Western diet rich in refined grains, cakes, sugar, processed red meat, fried foods, and butter has an opposite effect and increases the risk (31–33). Conceivably, the observed divergent relations of plasma choline and betaine with components of the metabolic syndrome could reflect diet if high intakes of meat and fruit or vegetables increase plasma choline and betaine, respectively. However, this possibility is not in agreement with the observations that betaine rather than choline was positively associated with total energy intake and that neither plasma choline nor betaine was positively related to the Western or Healthy dietary patterns. Furthermore, cholesterol, to a large degree derived from the intake of eggs, was the only nutrient that was associated with plasma choline, which showed no association with serum non-HDL cholesterol in a previous report from this study population (22). Taken together, these observations do not support the contention that the link between plasma choline and betaine and the metabolic syndrome reflects dietary intake.

The authors' responsibilities were as follows—Konstantinova: data collection, concept and design of the study, statistical analyses, data interpretation, and preparation of the first draft of the manuscript; Tell: planning, design, and concept of the study, data collection of the Hordaland Health Study and the Hordaland Health Study II, data interpretation, and critical revision of the manuscript; Vollset: concept and design of the study, statistical analyses, data interpretation, and critical revision of the manuscript; Ulvik: statistical analysis, data interpretation, and critical revision of the manuscript; Drevon: data interpretation and critical revision of the manuscript; and Ueland: concept and design of the study, data collection, data interpretation, and critical revision of the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES

1. Zeisel SH, Blusztajn JK. Choline and human nutrition. Annu Rev Nutr 1994;14:269–96.
2. Ueland PM, Holm PI, Hustad S. Betaine: a key modulator of one-carbon metabolism and homocysteine status. Clin Chem Lab Med 2005;43:1069–75.
3. Zeisel SH. Choline: critical role during fetal development and dietary requirements in adults. Annu Rev Nutr 2006;26:229–50.
4. Craig SAS. Betaine in human nutrition. Am J Clin Nutr 2004;80:539–49.
5. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. J Nutr 2003;133:1302–7.
6. Sakamoto A, Nishimura Y, Ono H, Sakura N. Betaine and homocysteine concentrations in foods. Pediatr Int 2002;44:409–13.
7. Detopoulou P, Panagiotakos DB, Antonopoulou S, Pitsavos C, Stefanadis C. Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. Am J Clin Nutr 2008;87:424–30.
8. Chiuve SE, Giovannucci EL, Hankinson SE, et al. The association between betaine and choline intakes and the plasma concentrations of homocysteine in women. Am J Clin Nutr 2007;86:1073–81.
9. Olthof MR, Brink EJ, Katan MB, Verhoef P. Choline supplemented as phosphatidylcholine decreases fasting and postmethionine-loading plasma homocysteine concentrations in healthy men. Am J Clin Nutr 2005;82:111–7.
10. Schwab U, Torronen A, Toppinen L, et al. Betaine supplementation decreases plasma homocysteine concentrations but does not affect body weight, body composition, or resting energy expenditure in human subjects. Am J Clin Nutr 2002;76:961–7.
11. Olthof MR, van Vliet T, Boelsma E, Verhoef P. Low dose betaine supplementation leads to immediate and long term lowering of plasma homocysteine in healthy men and women. J Nutr 2003;133:4135–8.
12. Atkinson W, Elmslie J, Lever M, Chambers ST, George PM. Dietary and supplementary betaine: acute effects on plasma betaine and homocysteine concentrations under standard and postmethionine load conditions in healthy male subjects. Am J Clin Nutr 2008;87:577–85.
13. Olthof MR, van Vliet T, Verhoef P, Zock PL, Katan MB. Effect of homocysteine-lowering nutrients on blood lipids: results from four randomised, placebo-controlled studies in healthy humans. PLoS MED 2005;2:446–56.
14. Olthof MR, Verhoef P. Effects of betaine intake on plasma homocysteine concentrations and consequences for health. Curr Drug Metab 2005;6:15–22.
15. Zeisel SH. Betaine supplementation and blood lipids: fact or artifact? Nutr Rev 2006;64:77–9.
16. Dalmeijer GW, Olthof MR, Verhoef P, Bots ML, van der Schouw YT. Prospective study on dietary intakes of folate, betaine, and choline and cardiovascular disease risk in women. Eur J Clin Nutr 2008;62:386–94.
17. Holm PI, Hustad S, Ueland PM, Vollset SE, Grotmol T, Schneede J. Modulation of the homocysteine-betaine relationship by methylenetetrahydrofolate reductase 677 C→T genotypes and B-vitamin status in a large-scale epidemiological study. J Clin Endocr Metab 2007;92:1535–41.
18. Holm PI, Ueland PM, Kvalheim G, Lien EA. Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-tandem mass spectrometry. Clin Chem 2003;49:286–94.
19. Holm PI, Ueland PM, Vollset SE, et al. Betaine and folate status as cooperative determinants of plasma homocysteine in humans. Arterioscler Thromb Vasc Biol 2005;25:379–85.
20. MelseBoonstra A, Holm PI, Ueland PM, Olthof M, Clarke R, Verhoef P. Betaine concentration as a determinant of fasting total homocysteine concentrations and the effect of folic acid supplementation on betaine concentrations. Am J Clin Nutr 2005;81:1378–82.
21. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005;365:1415–28.
22. Konstantinova SV, Tell GS, Vollset SE, Nygård O, Bleie Ø, Ueland PM. Divergent associations of plasma choline and betaine with components of the metabolic syndrome in middle age and elderly men and women. J Nutr 2008;138:914–20.
23. Andersen LF, Solvoll K, Johansson LRK, Salminen I, Aro A, Drevon CA. Evaluation of a food frequency questionnaire with weighed records, fatty acids, and alpha-tocopherol in adipose tissue and serum. Am J Epidemiol 1999;150:75–87.
24. Rimestad AH, Borgejordet Å, Vesterhus KN, et al. Den store matvaretabellen [The large food table]. Oslo, Norway: Gyldendal Undervisning, 2001 (in Norwegian).
25. Andersen LF, Nes M, Lillegaard IT, Sandstad B, Bjørneboe GE, Drevon CA. Evaluation of a quantitative food frequency questionnaire used in a group of Norwegian adolescents. Eur J Clin Nutr 1995;49:543–54.
26. Brevik A, Vollset SE, Tell GS, et al. Plasma concentration of folate as a biomarker for the intake of fruit and vegetables: the Hordaland Homocysteine Study. Am J Clin Nutr 2005;81:434–9.
27. Flood VM, Smith WT, Webb KL, Mitchell P. Issues in assessing the validity of nutrient data obtained from a food-frequency questionnaire: folate and vitamin B12 examples. Public Health Nutr 2004;7:751–6.
28. Willett W, Lenart E. Reproducibility and validity of food-frequency questionnaires. In: Willett W, ed. Nutritional epidemiology. 2nd ed. New York, NY: Oxford University Press, 1998:101–47.
29. Sweeney JP, Weihrauch JL. Summary of available data for cholesterol in foods and methods for its determination. CRC Crit Rev Food Sci Nutr 1976;8:131–59.
30. Welch RW. Cereal grains. In: Caballero B, Allen L, Prentice A, eds. Encyclopedia of human nutrition. 2nd ed. Oxford, United Kingdom: Elsevier Science, 2006:346-57.
31. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC. Dietary patterns, insulin resistance, and prevalence of the metabolic syndrome in women. Am J Clin Nutr 2007;85:910–8.
32. Feldeisen SE, Tucker KL. Nutritional strategies in the prevention and treatment of metabolic syndrome. Appl Physiol Nutr Metab 2007;32:46–60.
33. Lutsey PL, Steffen LM, Stevens J. Dietary intake and the development of the metabolic syndrome. The Atherosclerosis Risk in Communities Study. Circulation 2008;117:754–61.