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Research paper

Dietary choline is related to increased risk of acute myocardial infarction in patients with stable angina pectoris

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ABSTRACT

High plasma choline has been associated with the metabolic syndrome and risk of chronic diseases, including cardiovascular disease. However, dietary choline is not correlated with choline plasma concentrations, and there are few studies and contradictory evidence regarding dietary choline and cardiovascular events. In addition, a recommended dietary allowance for choline has not been established and remains a point of contention.

This study assessed the association between dietary choline, including choline forms, and risk of incident acute myocardial infarction (AMI) in patients with suspected stable angina pectoris (SAP).

In total 1981 patients (80% men, median age 62) from the Western Norway B Vitamin Intervention Trial were included in this analysis. Information on dietary choline was obtained using a 169-item food frequency questionnaire. The Cardiovascular Disease in Norway project provided data on AMI. Risk associations were estimated using Cox-regression analysis using energy-adjusted choline intake.

Median (25th, 75th percentile) total energy-adjusted choline intake was 288 (255, 326) mg/d. During a median (25th, 75th percentile) follow-up of 7.5 (6.3, 8.8) years, 312 (15.7%) patients experienced at least one AMI. Increased intakes of energy-adjusted choline (HR [95% CI] per 50 mg increase 1.11 [1.03, 1.20]), phosphatidylcholine (HR per 50 mg increase 1.24 [1.08, 1.42]) and sphingomyelin (HR per 5 mg increase 1.16 [1.02, 1.31]) were associated with higher AMI risk.

In conclusion, higher dietary intakes of total choline, phosphatidylcholine and sphingomyelin were associated with increased risk of AMI in patients with SAP. Future studies are necessary to explore underlying mechanisms for this observation.

1. Introduction

Choline is an essential nutrient, which to some extent can also be formed endogenously in the liver. Choline appears in both water- and lipid-soluble forms in both body and diet. Water-soluble forms include free choline, phosphocholine and glycerophosphocholine. They enter the liver via the portal circulation after intestinal choline transporter-mediated absorption [1,2]. Lipid-soluble forms include phosphatidylcholine (PC) and sphingomyelin (SM). PC is hydrolyzed by phospholipase A2 to lysoPC prior to absorption in the enterocyte [1,2]. LysoPC can be either reacetylated to PC or further broken down to glycerophosphocholine, and finally free choline [1,4]. PC enters the bloodstream through the lymphatic system incorporated in chylomicrons, thereby being delivered directly to peripheral tissue (muscle and

Abbreviations: AMI, acute myocardial infarction; CAD, coronary artery disease; CRP, C-reactive protein; CVD, cardiovascular disease; DMG, dimethylglycine; FFQ, food frequency questionnaire; MMA, methylmalonic acid; PC, phosphatidylcholine; PLP, pyridoxal phosphate; RCT, reverse cholesterol transport; SAP, stable angina pectoris; SM, sphingomyelin; TMAO, trimethylamine N-oxide; WENBIT, Western Norway B-Vitamin Intervention Trial.

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adipose) before reaching the liver [1]. SM is hydrolyzed by small intestinal brush border enzymes to ceramids and phosphocholine, and the latter is degraded to choline and transported by the portal vein to the liver [4]. Chemical structures of choline forms are provided in Supplementary Fig. 1.

The majority of choline in both diet and body is in the form of PC. Absorbed free choline is mainly used for PC formation via the Kennedy pathway, which is in its turn secreted into bile and very-low density lipoproteins (VLDL) [1,5]. The amount of PC in the bile largely exceeds the dietary supply (respectively 11 g/day vs 1–5 g/day). Approximately 95% of biliary PC is reabsorbed and 40% is returned to the liver, implying an extensive enterohepatic choline cycle [2]. Endogenous PC formation occurs via the phosphatidylethanolamine N-methyltransferase pathway, where PC is formed from phosphatidylethanolamine [1,2,5].

Dietary sources of choline are foods of animal origin like eggs, pork, beef, liver, milk, and plant sources like soybean and wheat germ [7]. Consumption of choline forms is dependent on the individual dietary pattern [1,2,8]. Unfortunately, data on choline content is not included in most food composition databases, including the Norwegian one [9]. There is no consensus so far on dietary choline requirements. While in the US the adequate intake is set to 550 mg/d for men and 425 mg/d for women, based on a few studies involving mostly men [10], values set in the European Union are slightly lower [11]. No reference values have been published specifically for the Nordic countries [12]. Mean choline intake in mainly North American and European countries, was below the dietary recommendation [13]. It is however not possible to conclude on the prevalence of choline intake deficiency since only an adequate intake has been established so far [13]. Additionally, few studies have reported intake of the individual choline forms [1,13], indicating the importance of evaluating choline intake in the population.

Choline is crucial for the synthesis of acetylcholine and major membrane phospholipids. Higher plasma choline has been associated with increased risk of cardiovascular disease (CVD) [5,10,14]. Choline's oxidation product, betaine, links choline to the one-carbon metabolism via its role in the betaine-homocysteine methyltransferase reaction where a methylgroup from betaine is transferred to homocysteine, forming methionine and dimethylglycine (DMG) [10,15]. Elevated plasma total homocysteine (tHcy) has been linked to increased risk of coronary artery disease (CAD) [16] and plasma DMG levels have been associated with risk of future acute myocardial infarction (AMI) [15] in the current population.

Only few and contradictory findings are published on dietary choline intake and CVD risk [14,17]. Additionally, previous studies have reported either no [7] or only marginal [14,18] correlations between plasma choline levels and dietary choline intake. So far, only egg intake has been linked to plasma choline levels in several studies [7,19]. In light of the few existing studies [17], it was the aim of this study to investigate the association between dietary choline, including choline forms, and subsequent risk of AMI in patients with stable angina pectoris (SAP).

2. Patients and methods

2.1. Study cohort

In total 3090 adult patients, undergoing elective coronary angiography due to suspected CAD between 1999 and 2004 at Haukeland University Hospital, Bergen and Stavanger University Hospital, Stavanger in Norway were enrolled in the Western Norway B Vitamin Intervention Trial (WENBIT, NCT00354081). This was a prospective, randomized, double-blind, placebo-controlled secondary prevention study that investigated the effect of vitamin B treatment on mortality and cardiovascular outcomes [20]. The study protocol has been described elsewhere [20]. For the current analysis, we included only patients with suspected SAP (n = 2573). We excluded patients with missing dietary data, including choline intake (n = 565), and those which reported extreme energy intake (i.e. <3000 kJ or >15 000 kJ for women and <3300 kJ or >17 500 kJ for men) (n = 27), which resulted in 1981 patients eligible for analyses.

The study was carried out according to the Declaration of Helsinki and approved by the Norwegian Data Inspectorate and the Regional Committee for Medical Health Research Ethics. All participants provided written informed consent.

2.2. Baseline data

Clinical information on patients' lifestyle and medical history was obtained from self-administered questionnaires or through interviews and verified by hospital records. Participants were defined as smokers based on self-reported smoking habits and serum cotinine levels >85 nmol/L at baseline [15]. Diabetes mellitus was defined according to preexisting diagnosis, HbA1c >6.5%, fasting blood glucose ≥7 mmol/L or non-fasting blood glucose ≥11.1 mmol/L according to the World Health Organization guidelines [21].

2.3. Follow-up and study end points

The primary end point of this study was incident AMI, including fatal and nonfatal events, classified according to the revised definition of AMI criteria (ICD-10 codes I21, I22, I46.1, R96, R98) [22]. Information on study outcomes was obtained from the Cardiovascular Disease in Norway (CVDNOR; https://cvdnor.b.uib.no) project, which reported on patients being discharged with a CVD diagnosis between 1994 and 2009 from 42 Norwegian public hospitals, and from the Cause of Death Registry at Statistics Norway (http://www.ssb.no).

2.4. Dietary assessment

Dietary data was obtained from a food frequency questionnaire (FFQ) given at the first visit and returned by email to the study center or at the one-month follow-up visit. The FFQ was an adaptation of a 180-item FFQ from 1992 developed at the Department of Nutrition, University of Oslo and designed to assess the habitual food intake of Norwegian adults. The adaptation resulted in a 169-food item FFQ designed to obtain information on the usual food intake over the past year. The frequency of consumption was given per day, week, month or never consumed depending on the food item. Portion sizes were given as household measures or units such as slices or pieces. Questions on the use of vitamin or mineral supplements were included, however, there were no questions regarding choline supplementation.

Nutrient intake was calculated using a database and software system developed at the Department of Nutrition, University of Oslo (Kostberegningssystem, version 3.2, University of Oslo, Norway). Intake of choline and individual choline forms was quantified using the U.S. Department of Agriculture (USDA) Database for Choline Content of Common Foods, release 2 [6]. Total dietary choline intake was estimated as the sum of free choline, PC, SM, phosphocholine and glycerophosphocholine. Choline content for food items that did not occur in the USDA database was estimated using nutritionally equivalent foods. For dishes that did not occur in the USDA database, choline content was calculated for each ingredient in the FFQ recipe.

2.5. Biochemical analyses

Routine biochemical analyses were conducted at the laboratories in the recruiting hospitals, whereas study-specific analyses were conducted by Bevital AS, Bergen, Norway (http://www.bevital.no). Choline compounds in plasma were analyzed by liquid chromatography-tandem mass spectrometry [23]. Details on the collection, storage and biochemical analysis of samples have been described previously [15].

2.6. Statistical analyses

Baseline variables and dietary intake are reported as median (25th, 75th percentile) for continuous variables or counts (percentages) for categorical variables. Patient baseline characteristics and dietary intake across quartiles of energy-adjusted choline intake were compared by median linear or logistic regression for continuous and categorical variables respectively. To adjust for reported energy intake the residual method was used for choline intake and nutrient density was calculated for macronutrients, food groups and specific food items [24].

Kaplan-Meier plots were used to visualize differences in survival across the quartiles of total choline intake, assessed by the log-rank test. Cox regression models were used to estimate the association between total choline intake and intake of choline forms and risk of AMI. The hazard ratios (HRs) and 95% confidence intervals (CI) were reported per daily increment of 50 mg for total choline and PC and of 5 mg for the remaining choline forms. The first model included reported energy intake, the second model additionally included age and sex and the final model additionally included smoking status. Traditional risk factors for CAD, fasting status, statin use and study intervention were not included in the model since they were not associated with choline intake and thus not considered being a confounder. Generalized additive models (GAMs) were plotted for the association between intakes of total choline and choline forms as continuous variables with AMI risk to explore non-linear relationships.

Effect modifications were studied according to subgroups of traditional risk factors for CAD, such as age, sex, body mass index, hypertension, smoking, diabetes mellitus, estimated glomerular filtration rate, baseline serum lipid parameters (including low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, apolipoprotein B (apoB) and apoA1) and to statin use at discharge and prior AMI. Continuous variables were dichotomized according to their median value and interactions were tested by adding interaction product terms with the continuous variable to the final cox regression model. Statistical analyses were performed using R version 3.4.3 (The R Foundation for Statistical Computing, Vienna, Austria), and the packages within the "*tidyverse*" *("dplyr", "ggplot2", "tidy", "broom", "purrr", "forcats", "tidyr")* [25], "*survival*" [26] and "*forestplot*" [27] were used for statistical analyses.

3. Results

3.1. Baseline characteristics

Baseline characteristics of the study patients (n = 1981) according to quartiles of energy-adjusted choline intake are presented in Table 1. The cohort consisted of 80% men, and the median (25th, 75th percentile) age was 62 (55, 69) years. In the total population, 28% were current smokers, 47% were diagnosed with hypertension, 31% had diabetes mellitus and 44% had a history of AMI. Moreover, choline intake was slightly inversely associated with plasma betaine, DMG and tHcy, but not associated with plasma concentrations of other one-carbon metabolites. A positive association was shown with plasma trimethylamine N-oxide (TMAO), plasma riboflavin, pyridoxal-5′-phosphate (PLP), and serum cobalamin and folate, whereas no association was observed with plasma methylmalonic acid (MMA).

3.2. Dietary choline intake

Dietary intake of choline and choline species, as well as nutrients and food groups across quartiles of energy-adjusted choline intake is shown in Table 2. The median (25th, 75th percentile) total energy intake was 2036 (1657, 2483) kcal/d and the energy-adjusted total choline intake was 288 (255, 326) mg/d. Total choline intake was mainly derived from PC (123 mg, 43%), followed by free choline (74 mg, 26%), glycerophosphocholine (63 mg, 22%), SM and phosphocholine (both 13 mg, 5%). Higher intake of total choline was inversely associated with intake of carbohydrates and positively associated with intake of fiber and protein. There was a slight increase in total fat, a decrease in saturated fatty acids (SFAs) and no change in intake of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) with increasing choline intake. Further, positive associations were found with intakes of cholesterol, alcohol, fruits and berries and vegetables. As expected, main dietary choline sources such as meat, fish, eggs and milk were all positively associated with choline intake.

3.3. Dietary choline intake and risk of AMI

During a median (25th, 75th percentile) follow-up time of 7.5 (6.3, 8.8) years, 312 (15.7%) patients experienced an AMI (Table 1). Fig. 1 depicts a Kaplan-Meier plot of event-free survival time across quartiles of energy-adjusted choline intake, showing a difference in event-free survival between the quartiles, with better survival in the first quartile and similar survival in the other three quartiles.

In a cox-model adjusted for energy intake only, we observed increased risk for AMI per 50 mg/d increment of total choline (HR 1.11, 95% CI [1.03, 1.20]) and PC (1.24 [1.08, 1.42]) intakes, and per 5 mg/d increment in SM (1.16 [1.02, 1.31]). These associations persisted after further adjustment for age and sex (model 2) and after additional adjustment for smoking (model 3) (Table 3). Intakes of free choline, phosphocholine or glycerophosphocholine intake were not associated with the risk of later AMI. Subgroup analyses showed no differences in AMI risk with regard to traditional risk factors for CAD (Supplementary Fig. 2).

The dose-response relationship between energy-adjusted choline intake and AMI is shown in Fig. 2. A positive linear relationship was observed for total choline, PC and SM whereas for free choline, phosphocholine and glycerophosphocholine there was no relationship.

4. Discussion

It was the aim of this study to investigate the association of dietary total choline and choline forms on future AMI risk in patients with suspected SAP. In this study, higher dietary choline intake, more specifically total choline, PC and SM, was associated with increased AMI risk during long-term follow-up, and the associations appeared to be linear across the intake ranges. The intakes of free choline, phosphocholine and glycerophosphocholine did not seem to be associated with AMI risk.

4.1. Previous studies on dietary choline and CVD risk

A systematic review and meta-analysis of four prospective studies on dietary choline and risk of incident CVD (defined as CAD, stroke or total CVD) reported no association (risk ratio 1.00)

Table 1
Patient characteristics according to quartiles of energy-adjusted daily choline intake.

Variable	Total cohort	Q1 (≤254)	Q2 (255–288)	Q3 (288–326)	Q4 (>326)	*P* trend
	(n = 1981)	(n = 496)	(n = 495)	(n = 495)	(n = 495)	
Total choline, mg/d	288 (255, 326)	232 (212, 244)	273 (264, 280)	306 (297, 315)	361 (341, 397)	<0.001
Incident AMI, n (%)	312 (15.7)	57 (11.5)	87 (17.6)	78 (15.8)	90 (18.2)	0.007
Age, y	62 (55, 69)	62 (55, 68)	63 (56, 70)	62 (55, 70)	61 (55, 67)	0.036
Male sex, n (%)	1588 (80.2)	429 (86.5)	375 (75.8)	384 (77.6)	400 (80.8)	<0.001
BMI, kg/m^2	26 (24, 28)	25 (23, 28)	26 (24, 28)	26 (24, 28)	26 (25, 29)	<0.001
Cardiovascular risk factors, n (%)						
Smokers	558 (28.2)	125 (25.2)	123 (24.8)	131 (26.5)	179 (36.2)	0.913
Hypertension	938 (47.3)	217 (43.8)	232 (46.9)	237 (47.9)	252 (50.9)	0.324
Diabetes mellitus	613 (30.9)	138 (27.8)	146 (29.5)	145 (29.3)	184 (37.2)	0.504
Cardiovascular history, n (%)						
Prior AMI	864 (43.6)	214 (43.1)	212 (42.8)	207 (41.8)	231 (46.7)	0.92
Prior CABG	285 (14.4)	73 (14.7)	70 (14.1)	65 (13.1)	77 (15.6)	0.796
Prior PCI	450 (22.7)	130 (26.2)	95 (19.2)	110 (22.2)	115 (23.2)	0.009
Medication use, n[a] (%)						
Statins	1769 (89.3)	442 (89.1)	436 (88.1)	445 (89.9)	446 (90.1)	0.609
ACE inhibitors	395 (19.9)	85 (17.1)	96 (19.4)	101 (20.4)	113 (22.8)	0.358
ARB	230 (11.6)	45 (9.1)	58 (11.7)	63 (12.7)	64 (12.9)	0.174
Aspirin	1784 (90.1)	464 (93.5)	446 (90.1)	434 (87.7)	440 (88.9)	0.049
β-Blockers	1533 (77.4)	395 (79.6)	378 (76.4)	383 (77.4)	377 (76.2)	0.214
Diuretics	181 (9.1)	34 (6.9)	48 (9.7)	55 (11.1)	44 (8.9)	0.106
CRP, mg/L	1.7 (0.8, 3.3)	1.7 (0.9, 3.2)	1.5 (0.7, 3.1)	1.5 (0.8, 3.1)	1.8 (0.9, 3.7)	0.977
eGFR, mL/min/1.73m^2	92 (82, 100)	92 (83, 100)	91 (80, 98)	92 (81, 100)	94 (83, 101)	0.059
Plasma levels of one-carbon metabolites, μmol/L						
Choline	9.5 (8.1, 11.2)	9.6 (8.2, 11.2)	9.5 (8.0, 11.2)	9.4 (8.1, 11.2)	9.4 (8.1, 11.4)	0.876
Betaine	39.2 (32.1, 48.0)	40.2 (33.4, 48.9)	39.3 (30.9, 46.8)	39.3 (33.1, 48.4)	38.2 (31.3, 46.7)	0.023
DMG	4.0 (3.3, 4.8)	4.1 (3.4, 4.9)	4.0 (3.2, 4.7)	4.0 (3.3, 5.0)	3.8 (3.2, 4.8)	0.001
Glycine	198 (175, 229)	203 (180, 237)	199 (175, 232)	199 (176, 229)	193 (169, 223)	0.001
Serine	94 (81, 107)	95 (82, 108)	94 (80, 109)	95 (83, 107)	92 (81, 105)	0.354
Methionine	26.6 (22.7, 32.2)	26.6 (22.9, 31.1)	26.2 (22.5, 31.9)	26.5 (22.5, 32.0)	27.3 (22.8, 33.3)	0.177
Total homocysteine	10.2 (8.6, 12.1)	10.4 (8.9, 12.7)	10.2 (8.5, 12.0)	10.2 (8.6, 12.1)	9.9 (8.5, 11.6)	0.004
Cystathionine	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)	0.2 (0.2, 0.4)	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)	0.860
Cysteine	286 (265, 308)	286 (264, 307)	286 (264, 309)	286 (266, 309)	287 (266, 308)	0.681
TMAO, μmol/L	5.7 (3.6, 9.4)	5.3 (3.5, 8.5)	5.6 (3.6, 8.2)	5.8 (3.9, 9.9)	6.1 (3.8, 10.9)	0.004
TML, μmol/L	0.7 (0.5, 0.9)	0.7 (0.5, 0.8)	0.7 (0.5, 0.9)	0.7 (0.5, 0.9)	0.7 (0.5, 0.9)	0.132
Plasma markers of B-vitamin status						
Riboflavin, nmol/L	11.1 (7.6, 17.5)	9.6 (6.6, 15.6)	11.2 (7.8, 17.6)	11.1 (7.8, 16.9)	12.2 (8.3, 19.1)	0.282
PLP, nmol/L	40.8 (29.6, 56.5)	37.9 (27.2, 51.9)	41.6 (29.6, 56.1)	40.4 (29.8, 56.4)	43.4 (32.5, 61.6)	0.001
Cobalamin, pmol/L	340 (260, 428)	317 (239, 399)	338 (259, 433)	344 (259, 435)	365 (286, 449)	0.042
Folate, nmol/L	10.0 (7.3, 14.4)	9.5 (7.0, 12.7)	9.7 (7.0, 13.9)	10.1 (7.6, 15.6)	10.8 (7.8, 15.4)	0.034
MMA, μmol/L	0.2 (0.1, 0.2)	0.2 (0.1, 0.2)	0.2 (0.1, 0.2)	0.2 (0.1, 0.2)	0.2 (0.1, 0.2)	0.158
Serum lipids, mmol/L						
Total cholesterol,	4.9 (4.2, 5.6)	4.8 (4.2, 5.5)	4.9 (4.2, 5.6)	4.8 (4.2, 5.7)	5.0 (4.3, 5.7)	0.496
LDL cholesterol,	2.9 (2.3, 3.6)	2.9 (2.3, 3.6)	2.9 (2.4, 3.5)	2.8 (2.3, 3.6)	2.9 (2.4, 3.7)	0.747
HDL cholesterol,	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.5)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	0.175
TG,	1.54 (1.1, 2.2)	1.6 (1.1, 2.2)	1.5 (1.1, 2.2)	1.5 (1.1, 2.1)	1.5 (1.1, 2.3)	0.529
ApoB, g/L	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.9 (0.7, 1.0)	0.423
ApoA1, g/L	1.3 (1.1, 1.4)	1.3 (1.1, 1.4)	1.3 (1.1, 1.4)	1.2 (1.1, 1.4)	1.3 (1.1, 1.4)	0.837

Continuous variables are presented as medians (25th,75th percentile) and categorical variables are reported as counts (%). Patient baseline characteristics across quartiles are compared by median linear (continuous variables) or logistic (categorical variables) regression. Dietary choline intake is energy-adjusted according to the residual method. ARB indicates angiotensin II receptor blockers; AMI, acute myocardial infarction; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; CABG, coronary artery bypass grafting; CRP, C-reactive protein; DMG, dimethylglycine; eGFR, estimated glomerular filtration rate; MMA, methylmalonic acid; PCI, percutaneous coronary intervention; PLP, pyridoxal phosphate; TG, triglycerides; TMAO, trimethylamine N-oxide; TML, trimethyllysine.

[a] At discharge from hospital.

between dietary total choline and risk of CVD [17]. The studies were, as opposed to the current investigation, performed in initially healthy populations, and no analysis of the individual dietary choline forms was provided [17]. The lack of analyses of choline in food items may be one reason for the low number of studies on choline intake. This calls for action for extended analysis of choline contents in foods.

Indeed, few studies have analyzed the intakes of individual choline forms in relation to CVD. Zheng et al. [28] reported a higher PC intake to be associated with increased risk of all-cause and CVD mortality among healthy American men and women, in particular among diabetic patients. As in our study, PC was the major source of dietary choline. However, the authors did not observe an association between PC intake and incident CVD and suggested that the effects of PC intake may be stronger on CVD prognosis than on CVD development. A Japanese population-based cohort study [29] found a positive association of SM, but not PC or total choline with cardiovascular mortality risk in healthy men. In animal studies, feeding SM to either LDLr KO mice [30] or apoE$^{-/-}$ mice [31] gave contradicting results on atherogenesis. Indeed, our finding of an increase in AMI risk with increasing SM intake is consistent with the findings of Nagata et al. [29], despite differences in choline sources in a typical Japanese diet compared to a Nordic diet [7].

4.2. Possible mechanisms

The underlying mechanisms for potential associations between dietary choline and AMI incidence remain elusive. Digestion of both PC and SM is thought to impair intestinal cholesterol absorption [4,32], thus affecting lipid metabolism. In clinical studies, PC and

Table 2
Daily dietary intake according to quartiles of energy-adjusted choline intake.

	Total cohort	Q1 (≤254)	Q2 (255–288)	Q3 (288–326)	Q4 (>326)	*P* trend
	(n = 1981)	(n = 496)	(n = 495)	(n = 495)	(n = 495)	
Total choline, mg/day	288 (255, 326)	232 (212, 244)	273 (264, 280)	306 (297, 315)	361 (341, 397)	<0.001
Choline forms, mg/d						
Free choline	74 (66, 85)	63 (56, 70)	72 (67, 78)	77 (71, 84)	89 (80, 104)	<0.001
PC	123 (103, 147)	97 (84, 110)	117 (104, 128)	134 (117, 151)	159 (137, 183)	<0.001
SM	13 (11, 15)	10 (9, 12)	12 (11, 14)	14 (12, 15)	16 (14, 19)	<0.001
Phosphocholine	13 (10, 16)	9 (7, 11)	12 (10, 14)	14 (11, 17)	17 (14, 21)	<0.001
Glycerophosphocholine	63 (49, 78)	46 (36, 56)	61 (50, 70)	68 (57, 79)	86 (69, 105)	<0.001
Betaine, mg	135 (105, 169)	144 (116, 180)	127 (99, 159)	133 (102, 166)	135 (108, 169)	<0.001
Energy, kcal	2036 (1657, 2483)	2173 (1809, 2604)	1903 (1547, 2345)	1963 (1581, 2385)	2093 (1692, 2564)	<0.001
Carbohydrates, E%	49.8 (45.5, 54.0)	51.4 (46.9, 55.3)	50.7 (47.4, 54.6)	49.6 (45.2, 53.5)	47.8 (43.3, 52.2)	<0.001
Fiber, g/1000 kcal	11.9 (10.0, 14.0)	11.4 (9.7, 13.0)	12.1 (10.2, 14.3)	11.9 (10.2, 14.2)	12.2 (9.9, 14.8)	<0.001
Protein, E%	16.7 (15.2, 18.4)	15.1 (13.9, 16.6)	16.3 (15.2, 17.8)	17.1 (15.8, 18.6)	18.4 (17.1, 20.0)	<0.001
Total fat, E%	31.2 (27.8, 35.0)	31.9 (28.4, 35.6)	30.7 (27.7, 34.5)	31.2 (27.9, 35.0)	31.1 (27.4, 34.7)	0.035
SFA, E%	11.6 (10.0, 13.3)	12.1 (10.4, 13.8)	11.5 (10.1, 13.0)	11.5 (10.0, 13.2)	11.3 (9.6, 13.1)	<0.001
MUFA, E%	10.2 (9.0, 11.6)	10.2 (9.0, 11.6)	10.1 (8.9, 11.4)	10.4 (8.9, 11.7)	10.3 (9.0, 11.6)	0.208
PUFA, E%	6.9 (5.8, 8.4)	6.9 (5.9, 8.6)	6.6 (5.6, 8.2)	6.9 (5.9, 8.2)	7.1 (5.7, 8.4)	0.016
Cholesterol, mg	278 (216, 359)	257 (195, 324)	255 (197, 327)	285 (217, 356)	338 (261, 420)	<0.001
Alcohol, E%	1.0 (0.0, 2.8)	0.5 (0.0, 2.2)	0.9 (0.0, 2.3)	1.2 (0.1, 2.9)	1.4 (0.2, 3.7)	<0.001
Meat, g/1000 kcal	53.3 (39.0, 68.9)	49.6 (35.9, 64.3)	52.3 (39.2, 67.8)	54.5 (40.2, 69.4)	56.5 (41.5, 73.0)	<0.001
Fish, g/1000 kcal	48.7 (33.6, 68.9)	39.6 (27.0, 58.6)	47.7 (33.8, 65.6)	50.1 (36.4, 67.9)	59.9 (42.0, 81.6)	<0.001
Eggs, g/1000 kcal	7.2 (4.0, 11.2)	4.4 (2.6, 7.0)	6.7 (4.1, 10.0)	8.7 (5.2, 12.6)	10.4 (6.8, 15.3)	<0.001
Milk, g/1000 kcal	117 (41, 193)	70 (21, 125)	112 (33, 179)	138 (68, 214)	170 (84, 249)	<0.001
Fruit and berries, g/1000 kcal	107.2 (66.4, 161.7)	98.3 (61.8, 140.3)	111.8 (70.2, 165.6)	110.4 (69.4, 172.8)	111.5 (63.3, 169.4)	0.005
Vegetables, g/1000 kcal	88.8 (57.5, 133.6)	68.7 (44.4, 98.4)	87.8 (58.4, 122.7)	96.6 (65.2, 146.5)	116.5 (72.5, 177.8)	<0.001
Corn products, g/1000 kcal	106.0 (86.1, 127.2)	116.9 (95.7, 135.8)	110.9 (91.0, 129.2)	104.1 (86.9, 126.2)	94.7 (74.7, 114.6)	<0.001

Continuous variables are presented as medians (25th,75th percentile. Dietary intake across quartiles were compared by median linear regression. Choline and choline forms are energy-adjusted according to the residual method. The nutrient density method was used for other nutrients, food groups and specific food items.
PC indicated phosphatidylcholine; SM, sphingomyelin.

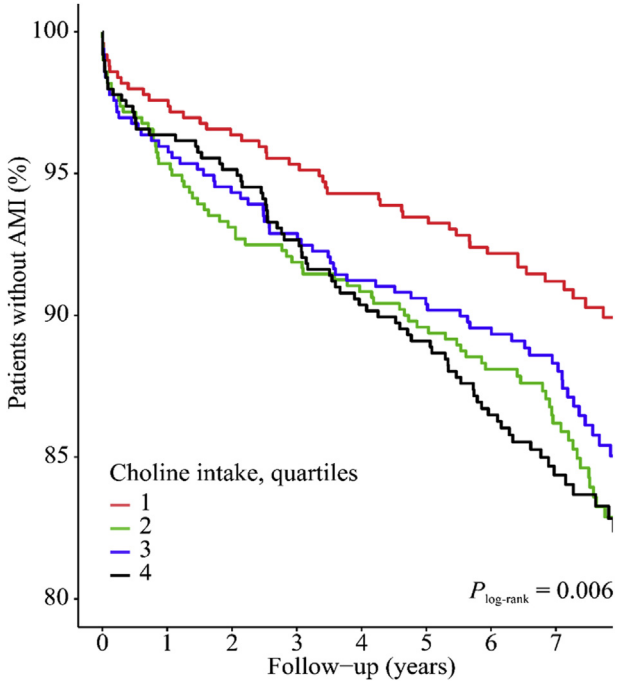

Fig. 1. Kaplan-Meier event-free survival curves for patients with choline intake in quartiles 1 to 4. A non-parametric log-rank test was used to compare survival between quartiles of energy-adjusted choline intake. The *x*-axis is trimmed at 7.5 years.
AMI indicates acute myocardial infarction.

SM from eggs is associated with beneficial changes in biomarkers related to reverse cholesterol transport and high-density lipoprotein characteristics [32,33], suggesting a favorable rather than a negative effect on atherosclerosis. At the same time, dietary choline, and choline-containing compounds such as PC, may exert negative effects through the conversion to trimethylamine by the intestinal microbiota, which is absorbed and transformed in the liver to TMAO by flavin-containing monooxygenase 3 (FMO3) [34]. Several studies, both in animals and humans, reported an association between dietary choline and TMAO formation, as well as a strong positive correlation between plasma TMAO concentration and cardiovascular events [34–37]. Indeed, we also observed increased TMAO levels at higher choline intakes, which may explain the association with increased CVD risk at least partly. Plasma TMAO might advance atherosclerosis by reduction of reverse cholesterol transport, increased macrophage cholesterol accumulation, upregulation of macrophage scavenger receptors and augmented foam cell formation, resulting in increased inflammation and low-density lipoprotein cholesterol oxidation [38,39]. A clear mechanistic link between circulating TMAO and CVD is, however, not yet validated. Additionally, circulating TMAO is not only influenced by diet, but also by the gut microbiome, FMO3 activity and excretion capacity [34,37], factors which were outside the scope of this study. Whether TMAO is a real contributor to atherosclerosis development or merely a marker of underlying pathogenic factors requires further research.

Second, PC is crucial for the formation of VLDL and its secretion from the liver [40]. The major fate of choline is conversion to PC and an estimated 70% of hepatic PC is made via the Kennedy pathway [2], however, to the best of our knowledge, there is no data on the amount of dietary choline incorporated into VLDL phospholipids. PC and other phospholipids produced in the Kennedy pathway may influence lipid metabolism through activation of peroxisome proliferator-activated receptor alpha [41]. In the current study, we found no baseline associations between choline intake and serum lipid parameters, nor did adjusting for such parameters alter the risk association between choline intake and later AMI. However,

Table 3
Hazard ratios for incident AMI according to energy-adjusted choline intake from cox regression analysis.

	Model 1[a]		Model 2[b]		Model 3[c]	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Total choline[d]	1.11 (1.03, 1.20)	0.006	1.12 (1.04, 1.21)	0.004	1.10 (1.02, 1.19)	0.015
Free choline[e]	1.02 (0.99, 1.05)	0.280	1.02 (0.99, 1.06)	0.180	1.02 (0.98, 1.05)	0.341
PC[d]	1.24 (1.08, 1.42)	0.002	1.25 (1.09, 1.44)	0.002	1.23 (1.07, 1.41)	0.003
SM[e]	1.16 (1.02, 1.31)	0.019	1.17 (1.04, 1.32)	0.011	1.15 (1.03, 1.30)	0.017
Phosphocholine[e]	1.06 (0.96, 1.18)	0.260	1.07 (0.96, 1.19)	0.203	1.07 (0.96, 1.19)	0.219
Glycerophosphocholine[e]	1.01 (0.99, 1.03)	0.251	1.01 (0.99, 1.03)	0.249	1.01 (0.99, 1.03)	0.249

HR indicates hazard ratio; CI, confidence interval; PC, phosphatidylcholine; SM, sphingomyelin.
[a] Adjusted for energy intake.
[b] Adjusted for energy intake, sex and age.
[c] Adjusted for energy intake, sex, age and smoking.
[d] per 50 mg per day increase.
[e] per 5 mg per day increase.

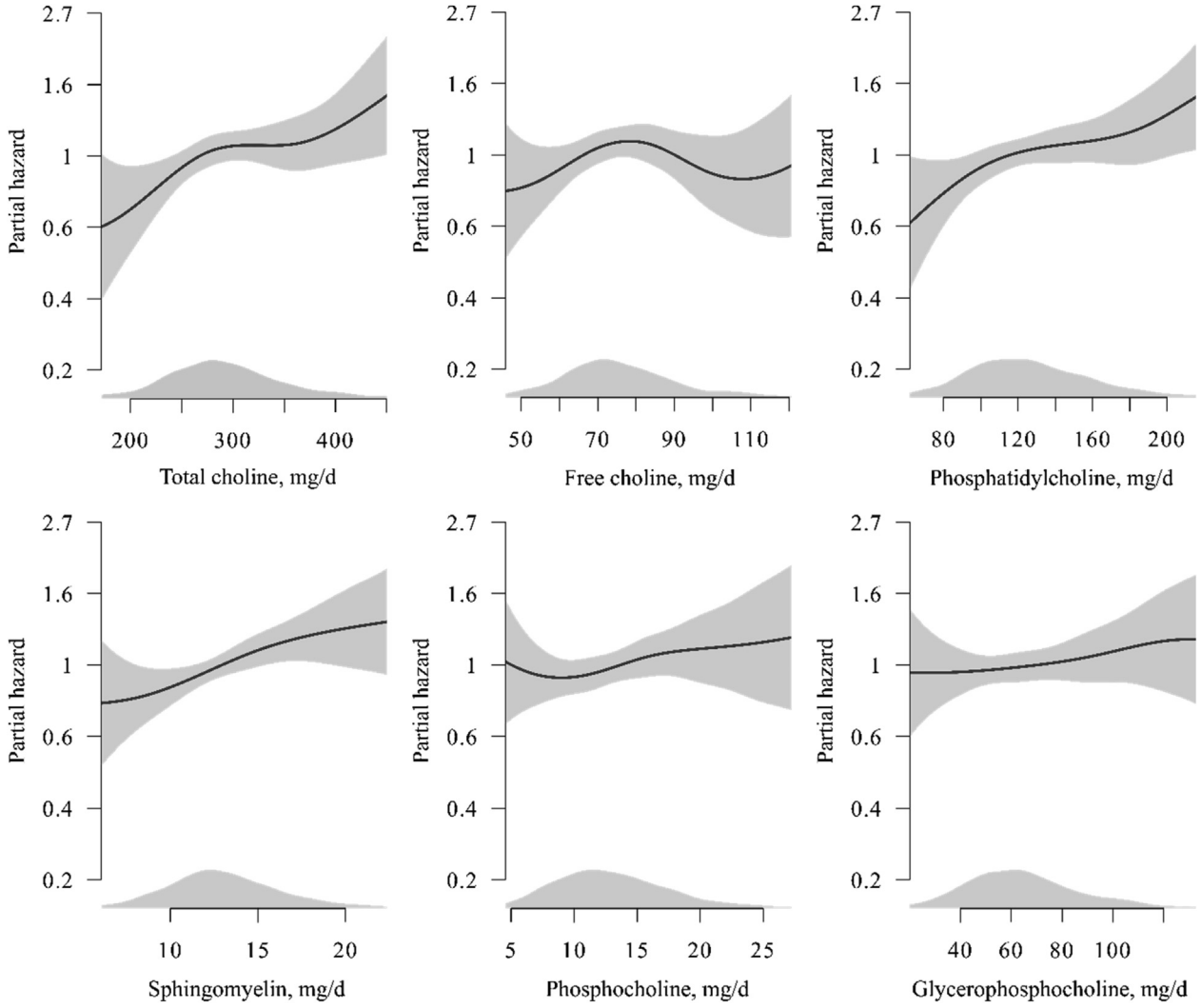

Fig. 2. Association of choline intake with risk of acute myocardial infarction using general additive models adjusted for total energy intake, sex, age and smoking. The solid lines show the observed association and the shaded areas 95% confidence intervals. Density plots indicate the distribution of dietary choline and choline forms.

any association between dietary choline and serum lipid parameters in our cohort might have been masked by high prevalence of statin use, although we did not find any interaction according to statin treatment in subgroup analyses. Nevertheless, our results deem it less likely that the choline-AMI risk relationship is explained by altered lipid levels per se.

Dietary choline intake was inversely associated with plasma tHcy concentrations in our cohort, being in line with several intervention studies showing that choline supplementation lowers plasma tHcy concentrations [42–45]. However, the causality of the association between homocysteine and AMI has been questioned and lowering of tHcy concentrations was not associated with reduced incidence of cardiovascular events in a meta-analysis of 8 randomized trials including 37 485 participants [46].

Also, dietary choline intake has been associated with lower inflammation markers such as CRP, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) in a cross sectional study involving healthy participants [47]. Pre-clinical studies showed potential anti-inflammatory effects of sphingolipids (with SM as main contributor) but the limited number of human studies and the use of complex phospholipid mixtures instead of single phospholipids, make it difficult to conclude on the effects of sphingolipids on inflammation [48]. In the present study, we did not observe any association between dietary choline and CRP. Il-6, TNF-α and neopterin were not investigated in our study.

The reported median energy-adjusted total choline intake was lower than the recommended intake [5,11]. This is in accordance with observational data from European [49,50], North-American [28,43,51,52] and Norwegian populations [14,18]. Since high choline intake was associated with AMI risk observed in this study and low choline intake has been associated with other adverse health outcomes, such as cancer, neurodegenerative diseases [5,10] and low bone mineral density [18], more research on health effects of choline intake is needed to define an adequate intake. The lack of correlation between dietary and plasma choline and a validated biomarker for choline intake [7,14,18], complicates this determination even more.

Increased PC and SM intake was associated with augmented AMI risk in our population. These fat soluble choline forms are mainly found in products of animal origin like eggs, beef, chicken, fish and milk [5]. Milk, meat and fish consumption increased gradually over the quartiles, while egg consumption in the highest quartile of total choline intake was 2.4 times higher compared to the lowest. Notably, these choline sources tend to have high cholesterol content (especially eggs) [12] and intake should be limited according to current dietary guidelines in context of general and cardiovascular health [12,53]. Additionally, eggs, processed and unprocessed meat are also high in carnitine, a TMAO precursor, and sodium (processed meat) which are associated with CVD risk via different pathways [54]. In contrast, a higher total choline intake was associated with higher vegetable and fibre intake, which are inversely associated with CVD risk [12]. Increased ingestion of plant-based food items result in increased intake of water-soluble choline forms, which were not associated AMI risk in our study. Thus, a high intake of the fat-soluble choline forms may be a marker of an otherwise unhealthy diet and therefore be associated with AMI risk. Importantly, findings for a nutrient are not necessarily valid for a food item containing that nutrient.

Even the association between eggs, which contain a high amount of PC and SM, and CVD remains controversial [54–56]. Further research is needed to explore the underlying mechanisms for the association between fat-soluble choline forms, the food items contributing to their intake and CVD risk.

4.3. Strengths and limitations

Among the strengths of the current study are the large sample size, the prospective design and the long-term follow-up. Detailed clinical and metabolic characterization of the population was available, and dietary intake of all choline forms and plasma concentration of choline were estimated. Additionally, the dietary analyses were adjusted for reported energy intake, which improves the accuracy of the estimates.

To the best of our knowledge, choline data in foods are only presented by the USDA database [6]. There is no data on choline content of Norwegian food items and there are difficulties in replacing local foods with foods included in the database that makes it impossible to exclude discrepancies. Next, the used FFQ was not validated for choline intake [57] and only filled out at baseline which makes it impossible to detect dietary changes over time. Additionally, random measurement error in estimated choline intake may have led to regression dilution bias and attenuated the relationship between choline intake and AMI. The ability to establish causality from this data is limited since it is impossible to exclude residual confounding.

5. Conclusion

In conclusion, increased intakes of total choline and choline forms PC and SM were associated with higher long-term AMI risk in patients with SAP. This is an important finding in light of the lower than recommended average intake in this cohort, and the widespread use of choline supplements. Therefore, future studies are warranted to explore underlying mechanisms for this association, as well as for improving dietary guidelines.

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Declaration of competing interest

No conflict of interest reported by any of the authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biochi.2019.11.001.

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