

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

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Running head: Dietary choline and future AMI risk

Abbreviations list:

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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1 **Abstract:**

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

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

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

15 Median (25<sup>th</sup>, 75<sup>th</sup> percentile) total energy-adjusted choline intake was 288 (255, 326) mg/d.  
16 During a median (25<sup>th</sup>, 75<sup>th</sup> percentile) follow-up of 7.5 (6.3, 8.8) years, 312 (15.7%) patients  
17 experienced at least one AMI. Increased intakes of energy-adjusted choline (HR [95% CI] per  
18 50 mg increase 1.11 [1.03, 1.20]), phosphatidylcholine (HR per 50 mg increase 1.24 [1.08,  
19 1.42]) and sphingomyelin (HR per 5 mg increase 1.16 [1.02, 1.31]) were associated with higher  
20 AMI risk.

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

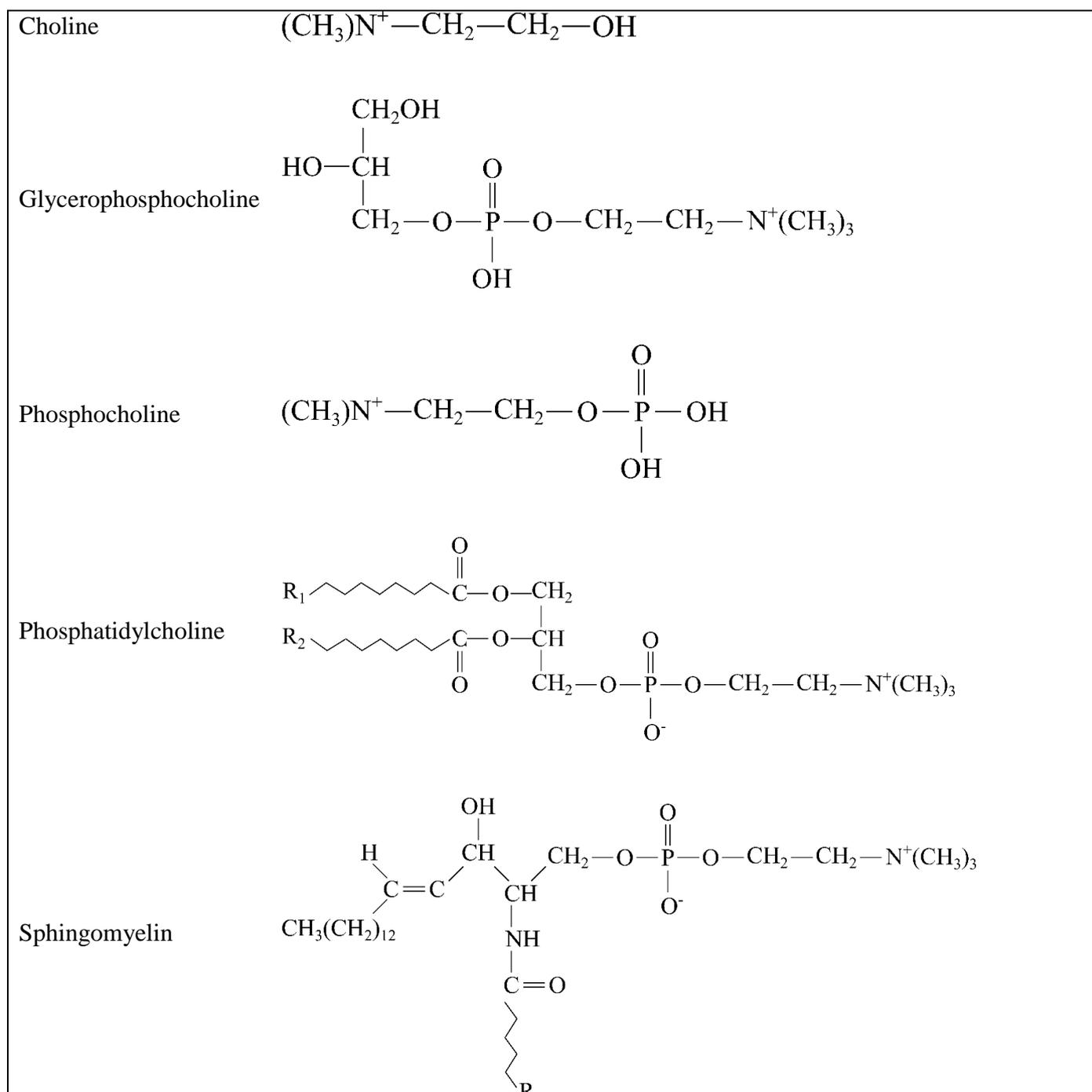
24 **Keywords**

25 Choline; Myocardial infarction; Phosphatidylcholine; Sphingomyelin; WENBIT

## 26 **1. Introduction**

27 Choline is an essential nutrient, which to some extent can also be formed endogenously in the  
28 liver. Choline appears in both water- and lipid-soluble forms in both body and diet. Water-  
29 soluble forms include free choline, phosphocholine and glycerophosphocholine. They enter the  
30 liver via the portal circulation after intestinal **choline transporter-mediated absorption** [1,2].  
31 Lipid-soluble forms include phosphatidylcholine (PC) and sphingomyelin (SM). **PC is**  
32 **hydrolyzed by phospholipase A2 to lysoPC prior to absorption in the enterocyte [1,2]. LysoPC**  
33 **can be either reacylated to PC or further broken down to glycerophosphocholine, and finally**  
34 **free choline [1,3]. PC enters the bloodstream through the lymphatic system incorporated in**  
35 **chylomicrons, thereby being delivered directly to peripheral tissue (muscle and adipose) before**  
36 **reaching the liver [1]. SM is hydrolyzed by small intestinal brush border enzymes to ceramids**  
37 **and phosphocholine, and the latter is degraded to choline and transported by the portal vein to**  
38 **the liver [3]. Chemical structures of choline forms are provided in **supplementary figure 1.****

39 **The majority of choline in both diet and body is in the form of PC. Absorbed free choline is**  
40 **mainly used for PC formation via the Kennedy pathway, which is in its turn secreted into bile**  
41 **and very-low density lipoproteins (VLDL) [1,4]. The amount of PC in the bile largely exceeds**  
42 **the dietary supply (respectively 11 g/day vs 1-5 g/day). Approximately 95% of biliary PC is**  
43 **reabsorbed and 40% is returned to the liver, implying an extensive enterohepatic choline cycle**  
44 **[2]. Endogenous PC formation occurs via the phosphatidylethanolamine N-methyltransferase**  
45 **pathway, where PC is formed from phosphatidylethanolamine [1,2,4].**



Supplementary figure 1: Chemical structures of choline and choline forms. Adapted from Patterson *et al.* [5].

46 Dietary sources of choline are foods of animal origin like eggs, pork, beef, liver, milk, and plant  
 47 sources like soybean and wheat germ [6]. Consumption of choline forms is dependent on the  
 48 individual dietary pattern [1,2,7]. Unfortunately, data on choline content is not included in most

49 food composition databases, including the Norwegian one [8]. There is no consensus so far on  
50 dietary choline requirements. While in the US the adequate intake is set to 550 mg/d for men  
51 and 425 mg/d for women, based on a few studies involving mostly men [9], values set in the  
52 European Union are slightly lower [10]. No reference values have been published specifically  
53 for the Nordic countries [11]. Mean choline intake in mainly North American and European  
54 countries, was below the dietary recommendation [12]. It is however not possible to conclude  
55 on the prevalence of choline intake deficiency since only an adequate intake has been  
56 established so far [12]. Additionally, few studies have reported intake of the individual choline  
57 forms [1,12], indicating the importance of evaluating choline intake in the population.

58 Choline is crucial for the synthesis of acetylcholine and major membrane phospholipids. Higher  
59 plasma choline has been associated with increased risk of cardiovascular disease (CVD)  
60 [4,9,13]. Choline's oxidation product, betaine, links choline to the one-carbon metabolism via  
61 its role in the betaine-homocysteine methyltransferase reaction where a methylgroup from  
62 betaine is transferred to homocysteine, forming methionine and dimethylglycine (DMG) [9,14].  
63 Elevated plasma total homocysteine (tHcy) has been linked to increased risk of coronary artery  
64 disease (CAD) [15] and plasma DMG levels have been associated with risk of future acute  
65 myocardial infarction (AMI) [14] in the current population.

66 Only few and contradictory findings are published on dietary choline intake and CVD risk  
67 [13,16]. Additionally, previous studies have reported either no [6] or only marginal [13,17]  
68 correlations between plasma choline levels and dietary choline intake. So far, only egg intake  
69 has been linked to plasma choline levels in several studies [6,18]. In light of the few existing  
70 studies [16], it was the aim of this study to investigate the association between dietary choline,  
71 including choline forms, and subsequent risk of AMI in patients with stable angina pectoris  
72 (SAP).

## 73 **2. Patients and methods**

### 74 **2.1 Study cohort**

75 In total 3090 adult patients, undergoing elective coronary angiography due to suspected CAD  
76 between 1999 and 2004 at Haukeland University Hospital, Bergen and Stavanger University  
77 Hospital, Stavanger in Norway were enrolled in the Western Norway B-Vitamin Intervention  
78 Trial (WENBIT, NCT00354081). This was a prospective, randomized, double-blind, placebo-  
79 controlled secondary prevention study that investigated the effect of vitamin B treatment on  
80 mortality and cardiovascular outcomes [19]. The study protocol has been described elsewhere  
81 [19]. For the current analysis, we included only patients with suspected SAP (n = 2573). We  
82 excluded patients with missing dietary data, including choline intake (n = 565), and those which  
83 reported extreme energy intake (i.e. <3000 kJ or >15 000 kJ for women and <3300 kJ or  
84 >17 500 kJ for men) (n = 27), which resulted in 1981 patients eligible for analyses.

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

### 88 **2.2 Baseline data**

89 Clinical information on patients' lifestyle and medical history was obtained from self-  
90 administered questionnaires or through interviews and verified by hospital records. Participants  
91 were defined as smokers based on self-reported smoking habits and serum cotinine levels >85  
92 nmol/L at baseline [14]. Diabetes mellitus was defined according to preexisting diagnosis,  
93 HbA1c >6.5%, fasting blood glucose  $\geq$ 7 mmol/L or non-fasting blood glucose  $\geq$ 11.1 mmol/L  
94 according to the World Health Organization guidelines [20].

### 95 **2.3 Follow-up and study end points**

96 The primary end point of this study was incident AMI, including fatal and nonfatal events,  
97 classified according to the revised definition of AMI criteria (ICD-10 codes I21, I22, I46.1,  
98 R96, R98) [21]. Information on study outcomes was obtained from the Cardiovascular Disease  
99 in Norway (CVDNOR; <https://cvdnor.b.uib.no>) project, which reported on patients being  
100 discharged with a CVD diagnosis between 1994 and 2009 from 42 Norwegian public hospitals,  
101 and from the Cause of Death Registry at Statistics Norway (<http://www.ssb.no>).

## 102 **2.4 Dietary assessment**

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

112 Nutrient intake was calculated using a database and software system developed at the  
113 Department of Nutrition, University of Oslo (Kostberegningssystem, version 3.2, University of  
114 Oslo, Norway). Intake of choline and individual choline forms was quantified using the U.S.  
115 Department of Agriculture (USDA) Database for Choline Content of Common Foods, release  
116 2 [5]. Total dietary choline intake was estimated as the sum of free choline, PC, SM,  
117 phosphocholine and glycerophosphocholine. Choline content for food items that did not occur  
118 in the USDA database was estimated using nutritionally equivalent foods. For dishes that did

119 not occur in the USDA database, choline content was calculated for each ingredient in the FFQ  
120 recipe.

## 121 **2.5 Biochemical analyses**

122 Routine biochemical analyses were conducted at the laboratories in the recruiting hospitals,  
123 whereas study-specific analyses were conducted by Bevital AS, Bergen, Norway  
124 (<http://www.bevital.no>). Choline compounds in plasma were analyzed by liquid  
125 chromatography-tandem mass spectrometry [22]. Details on the collection, storage and  
126 biochemical analysis of samples have been described previously [14].

## 127 **2.6 Statistical analyses**

128 Baseline variables and dietary intake are reported as median (25<sup>th</sup>, 75<sup>th</sup> percentile) for  
129 continuous variables or counts (percentages) for categorical variables. Patient baseline  
130 characteristics and dietary intake across quartiles of energy-adjusted choline intake were  
131 compared by median linear or logistic regression for continuous and categorical variables  
132 respectively. To adjust for reported energy intake the residual method was used for choline  
133 intake and nutrient density was calculated for macronutrients, food groups and specific food  
134 items [23].

135 Kaplan-Meier plots were used to visualize differences in survival across the quartiles of total  
136 choline intake, assessed by the log-rank test. Cox regression models were used to estimate the  
137 association between total choline intake and intake of choline forms and risk of AMI. The  
138 hazard ratios (HRs) and 95% confidence intervals (CI) were reported per daily increment of 50  
139 mg for total choline and PC and of 5 mg for the remaining choline forms. The first model  
140 included reported energy intake, the second model additionally included age and sex and the  
141 final model additionally included smoking status. Traditional risk factors for CAD, fasting  
142 status, statin use and study intervention were not included in the model since they were not

143 associated with choline intake and thus not considered being a confounder. Generalized additive  
144 models (GAMs) were plotted for the association between intakes of total choline and choline  
145 forms as continuous variables with AMI risk to explore non-linear relationships.

146 Effect modifications were studied according to subgroups of traditional risk factors for CAD,  
147 such as age, sex, body mass index, hypertension, smoking, diabetes mellitus, estimated  
148 glomerular filtration rate, baseline serum lipid parameters (including low-density lipoprotein  
149 cholesterol, high-density lipoprotein cholesterol, apolipoprotein B (apoB) and apoA1) and to  
150 statin use at discharge and prior AMI. Continuous variables were dichotomized according to  
151 their median value and interactions were tested by adding interaction product terms with the  
152 continuous variable to the final cox regression model.

153 Statistical analyses were performed using R version 3.4.3 (The R Foundation for Statistical  
154 Computing, Vienna, Austria), and the packages within the “tidyverse” (“dplyr”, “ggplot2”,  
155 “tidy”, “broom”, “purrr”, “forcats”, “tidyr”) [24], “survival” [25] and “forestplot” [26] was  
156 used for statistical analyses.

### 157 **3. Results**

#### 158 **3.1 Baseline characteristics**

159 Baseline characteristics of the study patients (n = 1981) according to quartiles of energy-  
160 adjusted choline intake are presented in **Table 1**. The cohort consisted of 80% men, and the  
161 median (25<sup>th</sup>, 75<sup>th</sup> percentile) age was 62 (55, 69) years. In the total population, 28% were  
162 current smokers, 47% were diagnosed with hypertension, 31% had diabetes mellitus and 44%  
163 had a history of AMI. Moreover, choline intake was slightly inversely associated with plasma  
164 betaine, DMG and tHcy, as well as with plasma trimethylamine N-oxide (TMAO), but not  
165 associated with plasma concentrations of other one-carbon metabolites. A positive association

166 was shown with plasma riboflavin, pyridoxal-5'-phosphate (PLP), and serum cobalamin and  
 167 folate, whereas no association was observed with plasma methylmalonic acid (MMA).

**TABLE 1**

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

Variable	Total cohort (n = 1981)	Q1 ( $\leq$ 254) (n = 496)	Q2 (255 - 288) (n = 495)	Q3 (288 - 326) (n = 495)	Q4 ( $>$ 326) (n = 495)	<i>P</i> trend
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 <sup>2</sup>	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 <sup>1</sup> (%)						
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
$\beta$ -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 <sup>2</sup>	92 (82, 100)	92 (83, 100)	91 (80, 98)	92 (81, 100)	94 (83, 101)	0.059
Plasma levels of one-carbon metabolites, $\mu$ mol/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, $\mu\text{mol/L}$	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, $\mu\text{mol/L}$	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, $\mu\text{mol/L}$	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 (25<sup>th</sup>, 75<sup>th</sup> 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.

<sup>1</sup> At discharge from hospital.

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.

## 168 **3.2 Dietary choline intake**

169 Dietary intake of choline and choline species, as well as nutrients and food groups across  
 170 quartiles of energy-adjusted choline intake is shown in **Table 2**. The median (25<sup>th</sup>, 75<sup>th</sup>  
 171 percentile) total energy intake was 2036 (1657, 2483) kcal/d and the energy-adjusted total  
 172 choline intake was 288 (255, 326) mg/d. Total choline intake was mainly derived from PC (123  
 173 mg, 43%), followed by free choline (74 mg, 26%), glycerophosphocholine (63 mg, 22%), SM

174 and phosphocholine (both 13 mg, 5%). Higher intake of total choline was inversely associated  
 175 with intake of carbohydrates and positively associated with intake of fiber and protein. There  
 176 was a slight increase in total fat, a decrease in saturated fatty acids (SFAs) and no change in  
 177 intake of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) with  
 178 increasing choline intake. Further, positive associations were found with intakes of cholesterol,  
 179 alcohol, fruits and berries and vegetables. As expected, main dietary choline sources such as  
 180 meat, fish, eggs and milk were all positively associated with choline intake.

**TABLE 2**

Daily dietary intake according to quartiles of energy-adjusted choline intake

	Total cohort (n = 1981)	Q1 ( $\leq 254$ ) (n = 496)	Q2 (255 - 288) (n = 495)	Q3 (288 - 326) (n = 495)	Q4 ( $> 326$ ) (n = 495)	<i>P</i> trend
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
Phosphocholin e	13 (10, 16)	9 (7, 11)	12 (10, 14)	14 (11, 17)	17 (14, 21)	<0.001
Glycerophosph ocholine	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/1000kcal	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

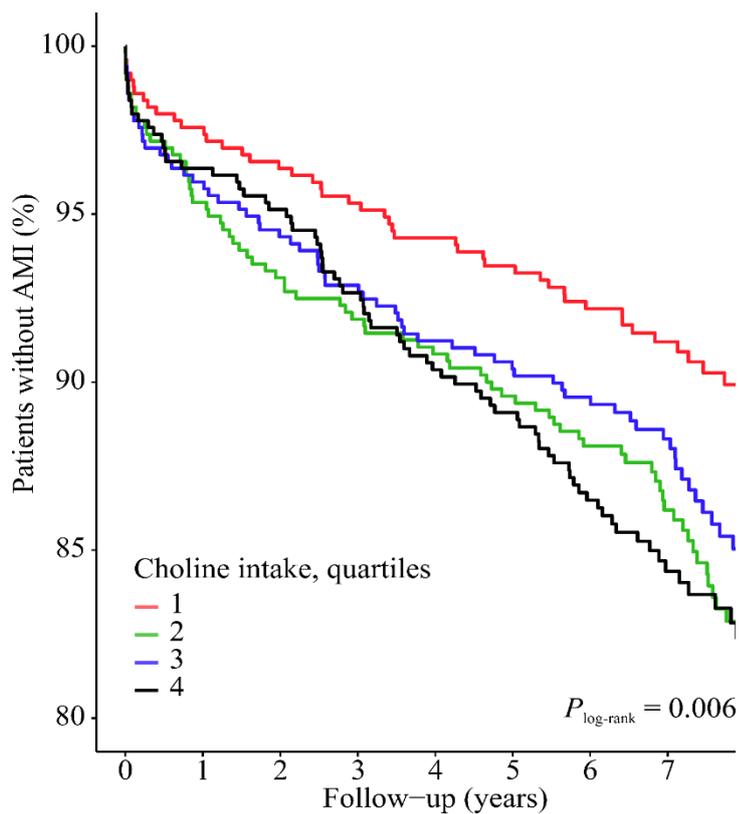
SFA, E%	11.6 (10.0, 13.3)	12.1 (10.4, 13.8)	11.5 (10.1, 13.0)	11.5 (10.0, 13.2)	11.3 (9.6, 13.1)	<0.001
MUFA, E%	10.2 (9.0, 11.6)	10.2 (9.0, 11.6)	10.1 (8.9, 11.4)	10.4 (8.9, 11.7)	10.3 (9.0, 11.6)	0.208
PUFA, E%	6.9 (5.8, 8.4)	6.9 (5.9, 8.6)	6.6 (5.6, 8.2)	6.9 (5.9, 8.2)	7.1 (5.7, 8.4)	0.016
Cholesterol, 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/1000kcal	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/1000kcal	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/1000kcal	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/1000kcal	117 (41, 193)	70 (21, 125)	112 (33, 179)	138 (68, 214)	170 (84, 249)	<0.001
Fruit and berries, g/1000kcal	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/1000kcal	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/ 1000kcal	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 (25<sup>th</sup>,75<sup>th</sup> 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.

### 181 3.3 Dietary choline intake and risk of AMI

182 During a median (25<sup>th</sup>, 75<sup>th</sup> percentile) follow-up time of 7.5 (6.3, 8.8) years, 312 (15.7%)  
 183 patients experienced an AMI (table 1). **Figure 1** depicts a Kaplan-Meier plot of event-free  
 184 survival time across quartiles of energy-adjusted choline intake, showing a difference in event-  
 185 free survival between the quartiles, with better survival in the first quartile and similar survival  
 186 in the other three quartiles.



187

Figure 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.

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

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

**TABLE 3**

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

	Model 1 <sup>1</sup>		Model 2 <sup>2</sup>		Model 3 <sup>3</sup>	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Total choline <sup>4</sup>	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 <sup>5</sup>	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 <sup>4</sup>	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 <sup>5</sup>	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 <sup>5</sup>	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 <sup>5</sup>	1.01 (0.99, 1.03)	0.251	1.01 (0.99, 1.03)	0.249	1.01 (0.99, 1.03)	0.249

<sup>1</sup> Adjusted for energy intake

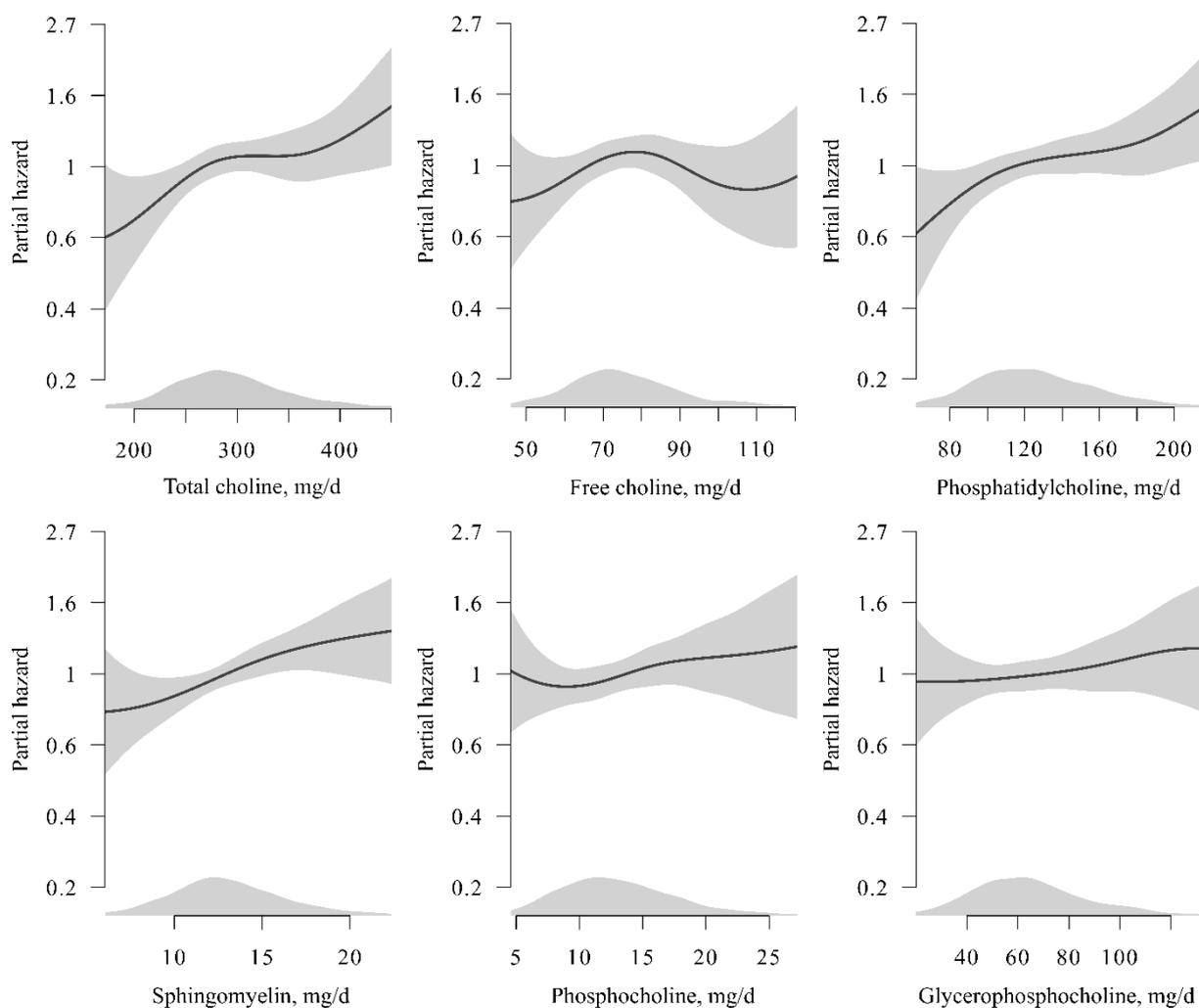
<sup>2</sup> Adjusted for energy intake, sex and age

<sup>3</sup> Adjusted for energy intake, sex, age and smoking

<sup>4</sup> per 50 mg per day increase

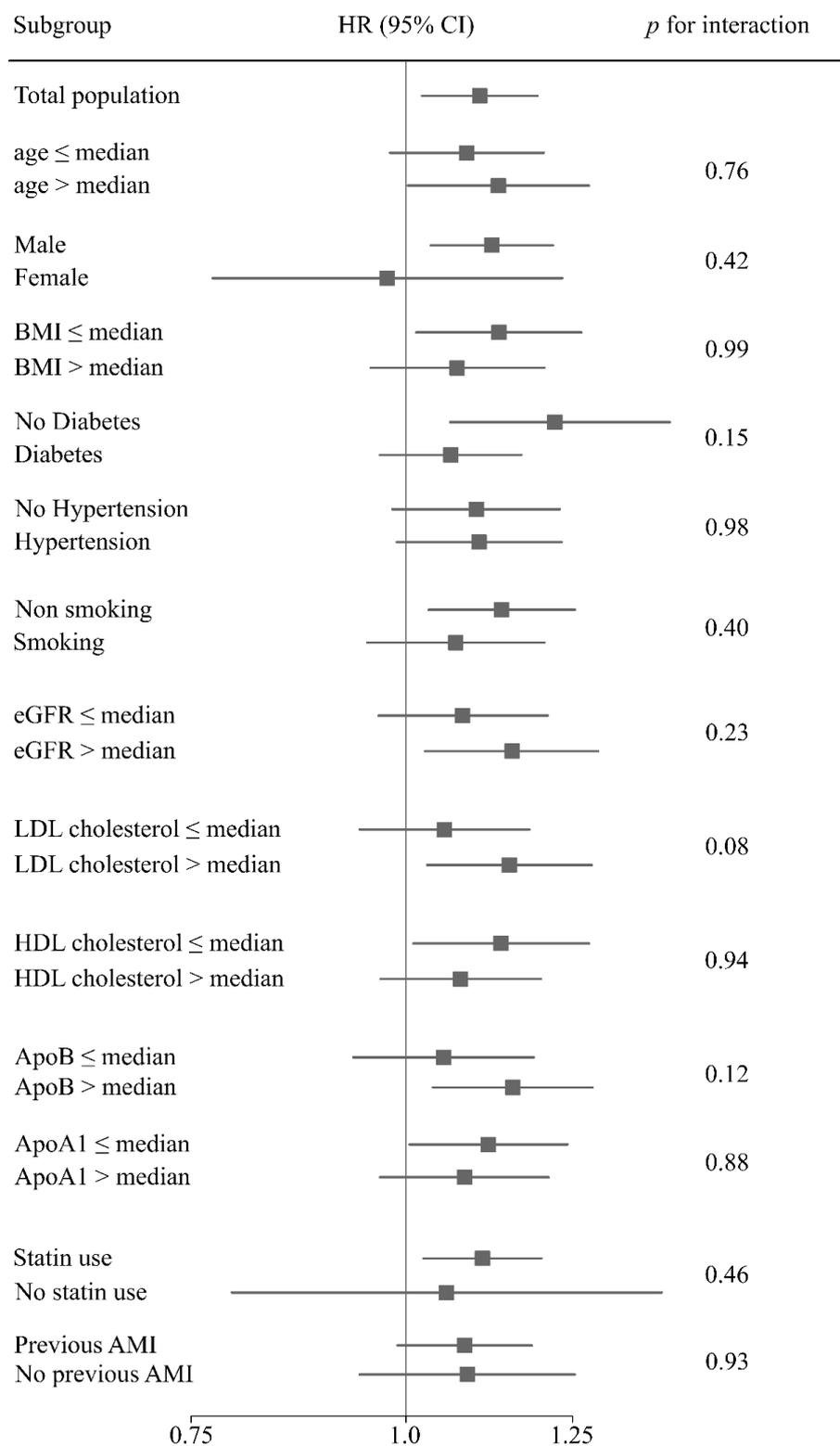
<sup>5</sup> per 5 mg per day increase

HR indicates hazard ratio; CI, confidence interval; PC, phosphatidylcholine; SM, sphingomyelin.



199

Figure 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.



Supplementary figure 2: Risk estimates per increment of 50 mg/d in total choline for incident AMI according to subgroups based on established risk factors for coronary artery disease based on a cox regression model adjusted for energy intake, sex, age and smoking. Continuous variables were dichotomized according to their median value and interactions

were tested statistically by adding interaction product terms with the non-dichotomized variable to the final cox model adjusted for total energy intake, sex, age and smoking.

AMI indicates acute myocardial infarction; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio.

## 201 **4. Discussion**

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

### 208 **4.1 Previous studies on dietary choline and CVD risk**

209 A systematic review and meta-analysis of four prospective studies on dietary choline and risk  
210 of incident CVD (defined as CAD, stroke or total CVD) reported no association (risk ratio 1.00)  
211 between dietary total choline and risk of CVD [16]. The studies were, **as opposed to the current**  
212 **investigation,** performed in initially healthy populations, and no analysis of the individual  
213 dietary choline forms was provided [16]. The lack of analyses of choline in food items may be  
214 one reason for the low number of studies on choline intake. This calls for action for extended  
215 analysis of choline contents in foods.

216 Indeed, few studies have analyzed the intakes of individual choline forms in relation to CVD.  
217 Zheng *et al.* [27] reported a higher PC intake to be associated with increased risk of all-cause  
218 and CVD mortality among healthy American men and women, in particular among diabetic  
219 patients. As in our study, PC was the major source of dietary choline. However, the authors did  
220 not observe an association between PC intake and incident CVD and suggested that the effects

221 of PC intake may be stronger on CVD prognosis than on CVD development. A Japanese  
222 population-based cohort study [28] found a positive association of SM, but not PC or total  
223 choline with cardiovascular mortality risk in healthy men. In animal studies, feeding SM to  
224 either LDLr KO mice [29] or apoE<sup>-/-</sup> mice [30] gave contradicting results on atherogenesis.  
225 Indeed, our finding of an increase in AMI risk with increasing SM intake is consistent with the  
226 findings of Nagata *et al.* [28], despite differences in choline sources in a typical Japanese diet  
227 compared to a Nordic diet [6].

#### 228 **4.2 Possible mechanisms**

229 The underlying mechanisms for potential associations between dietary choline and AMI  
230 incidence remain elusive. Digestion of both PC and SM is thought to impair intestinal  
231 cholesterol absorption [3,31], thus affecting lipid metabolism. In clinical studies, PC and SM  
232 from eggs is associated with beneficial changes in biomarkers related to reverse cholesterol  
233 transport and high-density lipoprotein characteristics [31,32], suggesting a favorable rather than  
234 a negative effect on atherosclerosis. At the same time, dietary choline, and choline-containing  
235 compounds such as PC, may exert negative effects through the conversion to trimethylamine  
236 by the intestinal microbiota, which is absorbed and transformed in the liver to TMAO by flavin-  
237 containing monooxygenase 3 (FMO3) [33]. Several studies, both in animals and humans,  
238 reported an association between dietary choline and TMAO formation, as well as a strong  
239 positive correlation between plasma TMAO concentration and cardiovascular events [33–36].  
240 Indeed, we also observed increased TMAO levels at higher choline intakes, which may explain  
241 the association with increased CVD risk at least partly. Plasma TMAO might advance  
242 atherosclerosis by reduction of reverse cholesterol transport, increased macrophage cholesterol  
243 accumulation, upregulation of macrophage scavenger receptors and augmented foam cell  
244 formation, resulting in increased inflammation and low-density lipoprotein cholesterol  
245 oxidation [37,38]. A clear mechanistic link between circulating TMAO and CVD is, however,

246 not yet validated. Additionally, circulating TMAO is not only influenced by diet, but also by  
247 the gut microbiome, FMO3 activity and excretion capacity [33,36], factors which were outside  
248 the scope of this study. Whether TMAO is a real contributor to atherosclerosis development or  
249 merely a marker of underlying pathogenic factors remains requires further research.

250 Second, PC is crucial for the formation of VLDL and its secretion from the liver [39]. The major  
251 fate of choline is conversion to PC and an estimated 70% of hepatic PC is made via the Kennedy  
252 pathway [2], however, to the best of our knowledge, there is no data on the amount of dietary  
253 choline incorporated into VLDL phospholipids. PC and other phospholipids produced in the  
254 Kennedy pathway may influence lipid metabolism through activation of peroxisome  
255 proliferator-activated receptor alpha [40]. In the current study, we found no baseline  
256 associations between choline intake and serum lipid parameters, nor did adjusting for such  
257 parameters alter the risk association between choline intake and later AMI. However, any  
258 association between dietary choline and serum lipid parameters in our cohort might have been  
259 masked by high prevalence of statin use, although we did not find any interaction according to  
260 statin treatment in subgroup analyses. Nevertheless, our results deem it less likely that the  
261 choline-AMI risk relationship is explained by altered lipid levels per se.

262 Dietary choline intake was inversely associated with plasma tHcy concentrations in our cohort,  
263 being in line with several intervention studies showing that choline supplementation lowers  
264 plasma tHcy concentrations [41–44]. However, the causality of the association between  
265 homocysteine and AMI has been questioned and lowering of tHcy concentrations was not  
266 associated with reduced incidence of cardiovascular events in a meta-analysis of 8 randomized  
267 trials including 37 485 participants [45].

268 Also, dietary choline intake has been associated with lower inflammation markers such as CRP,  
269 interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- $\alpha$ ) in a cross sectional study

270 involving healthy participants [46]. Pre-clinical studies showed potential anti-inflammatory  
271 effects of sphingolipids (with SM as main contributor) but the limited number of human studies  
272 and the use of complex phospholipid mixtures instead of single phospholipids, make it difficult  
273 to conclude on the effects of sphingolipids on inflammation [47]. In the present study, we did  
274 not observe any association between dietary choline and CRP. Il-6, TNF- $\alpha$  and neopterin were  
275 not investigated in our study.

276 The reported median energy-adjusted total choline intake was lower than the recommended  
277 intake [4,10]. This is in accordance with observational data from European [48,49], North-  
278 American [27,42,50,51] and Norwegian populations [13,17]. Since high choline intake was  
279 associated with AMI risk observed in this study and low choline intake has been associated  
280 with other adverse health outcomes, such as cancer, neurodegenerative diseases[4,9] and low  
281 bone mineral density [17], more research on health effects of choline intake is needed to define  
282 an adequate intake. The lack of correlation between dietary and plasma choline and a validated  
283 biomarker for choline intake [6,13,17], complicates this determination even more.

284 Increased PC and SM intake was associated with augmented AMI risk in our population. These  
285 fat soluble choline forms are mainly found in products of animal origin like eggs, beef, chicken,  
286 fish and milk [4]. Milk, meat and fish consumption increased gradually over the quartiles, while  
287 egg consumption in the highest quartile of total choline intake was 2.4 times higher compared  
288 to the lowest. Notably, these choline sources tend to have high cholesterol content (especially  
289 eggs) [11] and intake should be limited according to current dietary guidelines in context of  
290 general and cardiovascular health [11,52]. Additionally, eggs, processed and unprocessed meat  
291 are also high in carnitine, a TMAO precursor, and sodium (processed meat) which are  
292 associated with CVD risk via different pathways [53]. In contrast, a higher total choline intake  
293 was associated with higher vegetable and fibre intake, which are inversely associated with CVD  
294 risk [11]. Increased ingestion of plant-based food items result in increased intake of water-

295 soluble choline forms, which were not associated AMI risk in our study. Thus, a high intake of  
296 the fat-soluble choline forms may be a marker of an otherwise unhealthy diet and therefore be  
297 associated with AMI risk. Importantly, findings for a nutrient are not necessarily valid for a  
298 food item containing that nutrient.

299 Even the association between eggs, which contain a high amount of PC and SM, and CVD  
300 remains controversial [53–55]. Further research is needed to explore the underlying  
301 mechanisms for the association between fat-soluble choline forms, the food items contributing  
302 to their intake and CVD risk.

### 303 **4.3 Strengths and limitations**

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

309 Secondly, to the best of our knowledge, choline data in foods are only presented by the USDA  
310 database [5]. There is no data on choline content of Norwegian food items and there are  
311 difficulties in replacing local foods with foods included in the database that makes it impossible  
312 to exclude discrepancies. Next, the used FFQ was not validated for choline intake [56] and only  
313 filled out at baseline which makes it impossible to detect dietary changes over time.  
314 Additionally, random measurement error in estimated choline intake may have led to regression  
315 dilution bias and attenuated the relationship between choline intake and AMI. The ability to  
316 establish causality from this data is limited since it is impossible to exclude residual  
317 confounding.

### 318 **4.4 Conclusion**

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

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**Conflict of interest**

No conflict of interest reported by any of the authors.

## References

- [1] E.D. Lewis, C.J. Field, R.L. Jacobs, Should the forms of dietary choline also be considered when estimating dietary intake and the implications for health?, *Lipid Technol.* 27 (2015) 227–230. doi:10.1002/lite.201500048.
- [2] Z. Li, D.E. Vance, Phosphatidylcholine and choline homeostasis, *J. Lipid Res.* 49 (2008) 1187–1194. doi:10.1194/jlr.R700019-JLR200.
- [3] Å. Nilsson, R.-D. Duan, Pancreatic and mucosal enzymes in choline phospholipid digestion, *Am. J. Physiol. Liver Physiol.* 316 (2018) G425–G445. doi:10.1152/ajpgi.00320.2018.
- [4] S.H. Zeisel, K.C. Klatt, M.A. Caudill, Choline, *Adv. Nutr.* 9 (2018) 58–60. doi:10.1093/advances/nmx004.
- [5] K.Y. Patterson, S. a Bhagwat, J.R. Williams, J.C. Howe, J.M. Holden, USDA Database for the Choline Content of Common Foods, 2008. doi:10.15482/USDA.ADC/1178141.
- [6] S. V. Konstantinova, G.S. Tell, S.E. Vollset, A. Ulvik, C.A. Drevon, P.M. Ueland, Dietary patterns, food groups, and nutrients as predictors of plasma choline and betaine in middle-aged and elderly men and women, *Am. J. Clin. Nutr.* 88 (2008) 1663–1669. doi:10.3945/ajcn.2008.26531.
- [7] S.H. Zeisel, Dietary Choline : Biochemistry , Physiology , and Pharmacology, *Ann. Rev. Nutr.* 1 (1981) 95–121.
- [8] Norwegian Food Safety Authority, The Norwegian Directorate of Health University of Oslo, Norwegian Food Composition Database 2017, (2017). [www.matvaretabellen.no](http://www.matvaretabellen.no).
- [9] P.M. Ueland, Choline and betaine in health and disease, *J. Inherit. Metab. Dis.* 34 (2011) 3–15. doi:10.1007/s10545-010-9088-4.
- [10] EFSA (European Food Safety Authority), Dietary Reference Values for nutrients: Summary report, (2017) 49–50. doi:10.2903/sp.efsa.2017.e15121.
- [11] Nordic Council of Ministers, Nordic Nutrition Recommendations 2012: Integrating nutrition and physical activity, (2012).
- [12] A.M. Wiedeman, S.I. Barr, T.J. Green, Z. Xu, S.M. Innis, D.D. Kitts, Dietary choline intake: Current state of knowledge across the life cycle, *Nutrients.* 10 (2018). doi:10.3390/nu10101513.
- [13] H. Zuo, G.F.T. Svingen, G.S. Tell, P.M. Ueland, S.E. Vollset, E.R. Pedersen, A. Ulvik, K. Meyer, J.E. Nordrehaug, D.W.T. Nilsen, K.H. Bønaa, O. Nygård, Plasma concentrations and dietary intakes of choline and betaine in association with atrial

- fibrillation risk: Results from 3 prospective cohorts with different health profiles, *J. Am. Heart Assoc.* 7 (2018) 1–12. doi:10.1161/JAHA.117.008190.
- [14] G.F.T. Svingen, P.M. Ueland, E.K.R. Pedersen, H. Schartum-Hansen, R. Seifert, M. Ebbing, K.H. Løland, G.S. Tell, O. Nygård, Plasma dimethylglycine and risk of incident acute myocardial infarction in patients with stable angina pectoris, *Arterioscler. Thromb. Vasc. Biol.* 33 (2013) 2041–2048. doi:10.1161/ATVBAHA.113.301714.
- [15] L.L. Humphrey, R. Fu, K. Rogers, M. Freeman, M. Hefland, Homocysteine Level and Coronary Heart Disease Incidence : A Systematic Review and Meta-analysis, *Mayo Clin Proc.* 83 (2008) 1203–1212. doi:10.4065/83.11.1203.
- [16] K.A. Meyer, J.W. Shea, Dietary choline and betaine and risk of CVD: A systematic review and meta-analysis of prospective studies, *Nutrients.* 9 (2017). doi:10.3390/nu9070711.
- [17] J. Øyen, C.G. Gjesdal, T. Karlsson, G.F. Svingen, G.S. Tell, E. Strand, C.A. Drevon, K.J. Vinknes, K. Meyer, P.M. Ueland, O. Nygård, Dietary Choline Intake Is Directly Associated with Bone Mineral Density in the Hordaland Health Study, *J. Nutr.* 147 (2017) 572–578. doi:10.3945/jn.116.243006.
- [18] T.C. Wallace, V.L. Fulgoni, Usual choline intakes are associated with egg and protein food consumption in the United States, *Nutrients.* 9 (2017) 1–10. doi:10.3390/nu9080839.
- [19] M. Ebbing, Ø. Bleie, P.M. Ueland, J.E. Nordrehaug, D.W. Nilsen, S.E. Vollset, H. Refsum, E.K.R. Pedersen, O. Nygård, Mortality and Cardiovascular Events in Patients Treated With Homocysteine-Lowering, *JAMA.* 300 (2008) 795–804.
- [20] World Health Organization, Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation, (2006).
- [21] T.J.E.S. of C.A.C. of C. Committee, Myocardial infarction redefined - A consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction, *J. Am. Coll. Cardiol.* 36 (2000) 959–969. doi:10.1016/S0735-1097(00)00804-4.
- [22] P.I. Holm, P.M. Ueland, G. Kvalheim, E.A. Lien, Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-tandem mass spectrometry, *Clin. Chem.* 49 (2003) 286–294. doi:10.1373/49.2.286.
- [23] W.C. Willett, G.R. Howe, L.H. Kushi, Adjustment for total energy intake in

- epidemiologic studies, *Am J Clin Nutr.* 65 (1997) 1220S–8S.
- [24] H. Wickham, tidyverse: Easily Install and Load the “Tidyverse”., (2017). <https://cran.r-project.org/package=tidyverse>.
- [25] T. Therneau, A Package for Survival Analysis in S, (2015). <https://cran.r-project.org/package=survival>.
- [26] M. Gordon, T. Lunley, forestplot: Advanced Forest Plot Using “grid” Graphics, (2017). <https://cran.r-project.org/package=forestplot>.
- [27] Y. Zheng, Y. Li, E.B. Rimm, F.B. Hu, C.M. Albert, K.M. Rexrode, J.A.E. Manson, L. Qi, Dietary phosphatidylcholine and risk of all-cause and cardiovascular-specific mortality among US women and men, *Am. J. Clin. Nutr.* 104 (2016) 173–180. doi:10.3945/ajcn.116.131771.
- [28] C. Nagata, K. Wada, T. Tamura, K. Konishi, T. Kawachi, M. Tsuji, K. Nakamura, Choline and Betaine Intakes Are Not Associated with Cardiovascular Disease Mortality Risk in Japanese Men and Women, *J. Nutr.* 145 (2015) 1787–1792. doi:10.3945/jn.114.209296.
- [29] Z. Li, M.J. Basterr, T.K. Hailemariam, M.R. Hojjati, S. Lu, J. Liu, R. Liu, H. Zhou, X. Jiang, The effect of dietary sphingolipids on plasma sphingomyelin metabolism and atherosclerosis, *Biochim. Biophys. Acta.* 1735 (2005) 130–134. doi:10.1016/j.bbaliip.2005.05.004.
- [30] R.W.S. Chung, Z. Wang, C.A. Bursill, B.J. Wu, P.J. Barter, K. Rye, Effect of long-term dietary sphingomyelin supplementation on atherosclerosis in mice, *PLoS One.* (2017) 1–13.
- [31] C.N. Blesso, M.L. Fernandez, Dietary cholesterol, serum lipids, and heart disease: Are eggs working for or against you?, *Nutrients.* 10 (2018). doi:10.3390/nu10040426.
- [32] C.N. Blesso, Egg phospholipids and cardiovascular health, *Nutrients.* 7 (2015) 2731–2747. doi:10.3390/nu7042731.
- [33] C.E. Cho, M.A. Caudill, Trimethylamine- N -Oxide : Friend , Foe , or Simply Caught in the Cross-Fire ?, *Trends Endocrinol. Metab.* 28 (2017) 121–130. doi:10.1016/j.tem.2016.10.005.
- [34] W.W. Tang, Z. Wang, B.S. Levison, R.A. Koeth, E.B. Britt, X. Fu, Y. Wu, D. Ph, S.L. Hazen, Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk, *N. Engl. J. Med.* 368 (2013) 1575–1584. doi:10.1056/NEJMoa1109400.Intestinal.
- [35] M. Trøseid, T. Ueland, J.R. Hov, A. Svardal, I. Gregersen, C.P. Dahl, S. Aakhus, E.

- Gude, Microbiota-dependent metabolite trimethylamine-N-oxide is associated with disease severity and survival of patients with chronic heart failure, *J. Intern. Med.* 277 (2015) 717–726. doi:10.1111/joim.12328.
- [36] S.H. Zeisel, M. Warrier, Trimethylamine N -Oxide, the Microbiome, and Heart and Kidney Disease, *Annu. Rev. Nutr.* 37 (2017) 157–181. doi:10.1146/annurev-nutr-071816-064732.
- [37] Z. Wang, E. Klipfell, B.J. Bennett, R. Koeth, B.S. Levison, B. Dugar, A.E. Feldstein, E.B. Britt, X. Fu, Y.-M. Chung, Y. Wu, P. Schauer, J.D. Smith, H. Allayee, W.H.W. Tang, J.A. DiDonato, A.J. Lusis, S.L. Hazen, Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease, *Nature.* 472 (2011) 57–63. doi:10.1038/nature09922.Gut.
- [38] R. a Koeth, Z. Wang, B.S. Levison, J. a Buffa, E. Org, B.T. Sheehy, E.B. Britt, X. Fu, Y. Wu, J.D. Smith, J.A. Didonato, J. Chen, H. Li, G.D. Wu, J.D. Lewis, M. Warrier, J.M. Brown, R.M. Krauss, W.H.W. Tang, F.D. Bushman, A.J. Lusis, S.L. Hazen, Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis, *Nat Med.* 19 (2013) 576–585. doi:10.1038/nm.3145.Intestinal.
- [39] F. Gibellini, T.K. Smith, The Kennedy pathway-de novo synthesis of phosphatidylethanolamine and phosphatidylcholine, *IUBMB Life.* 62 (2010) 414–428. doi:10.1002/iub.337.
- [40] M. V Chakravarthy, I.J. Lodhi, L. Yin, R.R. V Malapaka, H.E. Xu, C.F. Semenkovich, Identification of a physiologically relevant endogenous ligand for PPAR $\alpha$  in liver, *Cell.* 138 (2009) 476–488. doi:10.1016/j.cell.2009.05.036.Identification.
- [41] R. Obeid, The Metabolic Burden of Methyl Donor Deficiency with Focus on the Betaine Homocysteine Methyltransferase Pathway, *Nutrients.* 5 (2013) 3481–3495. doi:10.3390/nu5093481.
- [42] E. Cho, S.H. Zeisel, P. Jacques, J. Selhub, L. Dougherty, Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framington Offspring Study, *Am J Clin Nutr.* 83 (2006) 905–911.
- [43] W. Atkinson, S. Slow, J. Elmslie, M. Lever, S.T. Chambers, P.M. George, Dietary and supplementary betaine : Effects on betaine and homocysteine concentrations in males, *Nutr. Metab. Cardiovasc. Dis.* 19 (2009) 767–773. doi:10.1016/j.numecd.2009.01.004.
- [44] M. Olthof, E. Brink, M. Katan, P. Verhoef, Choline supplemented as phosphatidylcholine decreases fasting and postmethionine-loading plasma homocysteine concentrations in healthy men, *Am J Clin Nutr.* 82 (2005) 111–117.

- [45] R. Clarke, J. Halsey, S. Lewington, E. Lonn, J. Armitage, J.E. Manson, K.H. Bo, D.J. Spence, O. Nygård, R. Jamison, M.J. Gaziano, P. Guarino, D. Bennett, F. Mir, R. Peto, R. Collins, Effects of Lowering Homocysteine Levels With B Vitamins on Cardiovascular Disease, Cancer, and Cause-Specific Mortality, *Arch Inter Med.* 170 (2010) 1622–1631.
- [46] P. Detopoulou, D.B. Panagiotakos, S. Antonopoulou, C. Pitsavos, C. Stefanadis, Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: The ATTICA study, *Am. J. Clin. Nutr.* 87 (2008) 424–430. doi:10.1093/ajcn/87.2.424.
- [47] G.H. Norris, C.N. Blesso, Dietary and endogenous sphingolipid metabolism in chronic inflammation, *Nutrients.* 9 (2017). doi:10.3390/nu9111180.
- [48] G.W. Dalmeijer, M.R. Olthof, P. Verhoef, M.L. Bots, Y.T. van der Schouw, Prospective study on dietary intakes of folate, betaine, and choline and cardiovascular disease risk in women, *Eur. J. Clin. Nutr.* 62 (2008) 386–394. doi:10.1038/sj.ejcn.1602725.
- [49] F.B.C. Vennemann, L.M. Valsta, C. Dumas, M.C. Ocké, G.B.M. Mensink, O. Lindtner, S.M. Virtanen, C. Tlustos, L.D. Addezio, I. Mattison, C. Dubuisson, I. Siksna, F. Héraud, Dietary intake and food sources of choline in European populations, *Br. J. Nutr.* (2015) 2046–2055. doi:10.1017/S0007114515003700.
- [50] M. Bertoia, J. Pai, J. Cooke, M. Joosten, M. Mittleman, E. Rimm, K. Mukamal, Plasma homocysteine, dietary B vitamins, betaine, and choline and risk of peripheral artery disease, *Atherosclerosis.* 235 (2014) 94–101. doi:10.1016/j.atherosclerosis.2014.04.010.Plasma.
- [51] A. Bidulescu, L.E. Chambless, A.M. Siega-Riz, S.H. Zeisel, G. Heiss, Usual choline and betaine dietary intake and incident coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) Study, *BMC Cardiovasc. Disord.* 7 (2007) 1–8. doi:10.1186/1471-2261-7-20.
- [52] R.H. Eckel, J.M. Jakicic, J.D. Ard, J.M. De Jesus, N.H. Miller, V.S. Hubbard, I. Lee, A.H. Lichtenstein, C.M. Loria, B.E. Millen, C.A. Nonas, F.M. Sacks, S.C. Smith, L.P. Svetkey, T.A. Wadden, S.Z. Yanovski, K.A. Kendall, L.C. Morgan, M.G. Trisolini, G. Velasco, J. Wnek, J.L. Anderson, J.L. Halperin, N.M. Albert, B. Bozkurt, R.G. Brindis, L.H. Curtis, D. Demets, J.S. Hochman, R.J. Kovacs, E.M. Ohman, S.J. Pressler, F.W. Sellke, W. Shen, AHA/ACC guidelines on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart

- Association Task Force on Practice Guidelines, *Circulation*. 129 (2014) 76–99.  
doi:10.1331/JAPhA.2014.14501.
- [53] V.W. Zhong, L. Van Horn, M.C. Cornelis, J.T. Wilkins, H. Ning, M.R. Carnethon, P. Greenland, R.J. Mentz, K.L. Tucker, L. Zhao, A.F. Norwood, D.M. Lloyd-Jones, N.B. Allen, Associations of Dietary Cholesterol or Egg Consumption with Incident Cardiovascular Disease and Mortality, *JAMA - J. Am. Med. Assoc.* 321 (2019) 1081–1095. doi:10.1001/jama.2019.1572.
- [54] C. Qin, J. Lv, Y. Guo, Z. Bian, J. Si, L. Yang, Y. Chen, Y. Zhou, H. Zhang, J. Liu, J. Chen, Z. Chen, C. Yu, L. Li, Associations of egg consumption with cardiovascular disease in a cohort study of 0.5 million Chinese adults, *Heart*. 104 (2018) 1756–1763. doi:10.1136/heartjnl-2017-312651.
- [55] Z.S. Clayton, E. Fusco, M. Kern, Egg consumption and heart health: A review, *Nutrition*. 37 (2017) 79–85. doi:10.1016/j.nut.2016.12.014.
- [56] L.F. Andersen, K. Solvoll, L.R.K. Johansson, I. Salminen, A. Aro, C.A. Drevon, Evaluation of a Food Frequency Questionnaire with Weighed Records, Fatty Acids, and Alpha-Tocopherol in Adipose Tissue and Serum, *Am. J. Epidemiol.* 150 (1999) 75–87. doi:10.1093/oxfordjournals.aje.a009921.