

Choline intake and Incidence of Acute Myocardial Infarction in Patients with Stable Angina Pectoris

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Preface

Above all, I thank the Source of all things for having supplied me with all the strength and all the necessary to initiate, endure and conclude this project.

My dear counsellors Ottar Nygård, Vegard Lysne, and specially Therese Karlsson, many thanks for your time, your energy and for not giving up on me. Your help has been invaluable important to me.

My Dahl, thank you, my love! Thank you for your support and patience. Thank you for being by my side.

My eternal thankfulness to my beloved family: my mother Deise, my brother Leandro and my sister Renata who has fought beside me and provided me with courage and strength. You were there when nobody else was, you cried my tears and fought my fights. I love you!

To the Andreassen family my most sincere thanks. You have given me invaluable help by taking care of the most precious person in my life: Rebecca Palma Andreassen.

Bekinha Palma, you have inspired me day and night. You have given me your love and your support without knowing it, my child, and now I offer you the goods of my work. I will always be there for you. I love you unconditionally.

And finally, I offer this work in memory of my father, Cidio Alves Palma, my hero. I will always love you, father.

Summary

Introduction Cardiovascular diseases are the leading cause of death worldwide. It can be defined as a group of interrelated diseases of the heart and blood vessels, including atherosclerotic cardiovascular diseases. Acute myocardial infarction (AMI) may be the first manifestation of coronary heart disease (CHD) or it may develop during more chronic stages of CHD. The major risk factors for CHD are tobacco use, unhealthy diet, obesity, physical inactivity, hypertension, diabetes, and hyperlipidemia. Thus, life style changes affecting these risk factors are important in primary and secondary prevention of CHD.

A high CHD risk diet comprises high intakes of fat, refined sugar, meat and low intake of fruits and vegetables. The essential nutrient choline, which is the focus of the current study, is found in virtually all foods, but meat and other animal products are the main sources of dietary choline among omnivorous populations. These are food items that, in accordance with current dietary guidelines, should be limited in our diet. Intak of choline has been linked to increased AMI risk. However, few studies have investigated the association between choline intake and risk of AMI in patients with established CHD.

Objective To analyse a possible association between choline intake and risk of AMI in patients with suspected stable angina pectoris.

Methods We used data from 2019 patients from the Western Norway B-Vitamin Intervention Trial who underwent coronary angiography at baseline. Average food consumption for the previous year was collected via a 169-item food frequency questionnaire at baseline. Total intakes of choline and choline species were adjusted by total energy intake by using the residual method. For continuous variables, we used Students T-test to analyze differences between patients who developed AMI and those who did not, and linear regression to explore trends across quartiles of total choline intake. For dichotomous and categorical variables logistic regression and Fisher's exact test were used, respectively.

For estimating the risk of AMI, Cox proportional hazards regression models were used. Hazard ratios and confidence intervals are presented per 100 mg increase in total choline intake and for each 10 mg increase in free choline, phosphatidylcholine, phosphocholine, sphingomyelin and glycerophosphocholine. Three models were tested to control for confounders on the effect of dietary choline on risk of AMI. Finally, potential non-linear

associations between choline intake and risk of AMI were explored using general additive models.

Results Mean (SD) daily total choline intake among the 2019 participants was 294 (65.1) mg (79.7% were men, mean age was 61.8 (9.7) years). No significant association between choline intake and sex, age, prior CVD or extent of coronary artery disease (CAD) at baseline. Higher choline intake was however positively associated with several established CVD risk factors including smoking ($p < 0.001$), BMI ($p < 0.001$), hypertension ($p < 0.005$), diabetes ($p < 0.001$), serum glucose ($p < 0.001$), but inversely associated with plasma total homocysteine (Hcy) ($p < 0.001$). No association was observed with lipid related parameters. During a median follow up of 7.2 (2.4) years, 297 patients experienced an AMI. In the crude model, adjusted for total energy intake, the risk of AMI increased with 28% (CI 1.09-1.49) for each 100 mg increase in choline intake. Model 2 was also adjusted for sex, age, smoking, previous AMI, previous coronary artery bypass grafting (CABG) and extension of CAD at baseline. Model 3 was further adjusted for BMI and diabetes. In the multivariate models the risk was slightly attenuated. Intake of phosphatidylcholine and sphingomyelin was positively associated with risk of AMI, whereas intake of free choline, phosphocholine and glycerophosphocholine showed no association with AMI risk.

Conclusion In patients with SAP, a higher intake of choline is associated with a number of established risk factors for CVD but with independent excess risk of AMI.

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Abbreviation list

5-MTHF – 5-methyltetrahydrofolate

ACEI/ARB - Angiotensin-converting enzyme inhibitor and Angiotensin receptor blocker

ACS – Acute Coronary Syndrome

AI – Adequate Intake

AMI – Acute Myocardial Infarction

BHMT – Betaine-Homocysteine Methyltransferase

BMI – Body Mass Index

CABG – Coronary Artery Bypass Grafting

CAD – Coronary Artery Disease

CDP-choline – Cytidine Diphosphocholine

CHD – Coronary Heart Disease

CI – Confidence Interval

CRP – C-Reactive Protein

CVD – Cardiovascular Disease

DMG – Dimethylglycine

EFSA – European Food Safety Authority

FFQ – Food Frequency Questionnaire

FMO – Flavin Monooxygenase

FMO 3 – Flavin Monooxygenase 3

GAM – General Additive Model

Hcy – Homocysteine

HDL-C – High-Density Lipoprotein Cholesterol

HR – Hazard Ratio

IOM – Institute of Medicine

LDL-C – Low-Density Lipoprotein Cholesterol

LVEF – Left Ventricular Ejection Fraction

MET – Methionine

MS – Methionine Synthase

MTHFR – Methylene tetrahydrofolate Reductase

MUFA – Monounsaturated Fatty Acid

NNR – Nordic Nutrition Recommendations

PCI – Percutaneous Coronary Intervention

PEMT – Phosphatidylethanolamine-*N*-TransferaseMS
PUFA – Polyunsaturated Fatty Acid
SAH – S-Adenosylhomocysteine
SAM – S-Adenosylmethionine
SAP – Stable Angina Pectoris
SD – Standard Deviation
SFA – Saturated Fatty Acid
SNP – Single Nucleotide Polymorphism
tHcy – Total Homocysteine
TFA – Trans Fatty Acid
TG – Triglycerides
TMA - Trimethylamine
TMAO – Trimethylamine N-Oxide
UL – Tolerable Upper Intake Level
USDA – US Department of Agriculture
VLDL-C – Very-Low Density Lipoprotein Cholesterol

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1. Introduction

1.1 Cardiovascular Disease

1.1.1 Background

Cardiovascular disease (CVD) is a group of diseases of the heart and blood vessels that are interrelated. Within this group, we find atherosclerotic CVDs that include coronary heart disease (CHD), diseases of the aorta, cerebrovascular disease and diseases of the peripheral arteries.

CVD is a noncommunicable disease responsible for over 4 million deaths each year among Europeans, an estimated 45% of all deaths. Seventy percent of those CVD deaths are due to CHD (1.8 million) and cerebrovascular disease (1.0 million). In addition, more women than men die of CVD, 2.2 million (49% of all deaths) against 1.8 million (40% of all deaths), respectively (Townsend et al, 2016). CVD is still the number one death cause in Europe (Townsend et al., 2016), and worldwide (Rajaie and Esmailzadeh, 2011), having caused 17.3 million deaths or 31.5% of all deaths globally in 2013 (Townsend et al., 2016). Despite constant and significant efforts to combat disease, CVD grows in importance globally, especially in low- and middle-income countries (Mendis *et al.*, 2011).

1.1.2 Acute Myocardial Infarction

CHD may manifest as stable angina pectoris (SAP), unstable angina pectoris, acute myocardial infarction (AMI), heart failure and sudden death (Hinchliffe and Green, 2014; Mendis *et al.*, 2011). AMI may be the first manifestation of CHD or it may develop in patients already treated for established CHD (Thygesen et al., 2012). An incident AMI is usually defined as the first AMI in a subject, but in this study it will be defined as the first AMI developed during follow-up of the patients.

An AMI usually develops secondary to progression atherosclerosis (Mendis et al., 2011; Fox et al., 2006) and after an individual experiences the first AMI event, there is a higher risk to experience AMI in the future (Mendis et al., 2011; Thygesen et al., 2012).

1.1.3 Stable Angina Pectoris

According to the European Society of Cardiology guidelines (Fox et al., 2006), SAP affects around 0.1 – 1% of women between 45 and 54 years old, and 10 – 15% of women between 65 and 74 years old. Among 45 to 54-year-old men, the prevalence is around 2 – 5%, and in men aged 65 to 74 years old, prevalence is 10 – 20%.

SAP is a clinical syndrome characterized by chest pain or shortness of breath (Fox et al., 2006). When these symptoms are caused by myocardial ischemia is usually due to underlying obstructive atherosclerotic disease in the coronary arteries. The diagnosis is usually referred to as suspected SAP if the status of the coronary arteries is unknown.

1.1.4 Established Risk Factors

The established risk factors for CHDs include tobacco use, unhealthy diet, physical inactivity, obesity, hypertension, diabetes and hyperlipidaemia (Liu et al., 2000; Mendis *et. al.*, 2011; Hames, 2014), and excessive use of alcohol (Raymond and Couch, 2012). Although most studies use total cholesterol, low density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) together with triglycerides (TG) as parameters of lipid status, measuring the concentrations of the main apolipoproteins of the LDL-C particle (apolipoprotein B – ApoB) and HDL-C particle (Apolipoprotein A1 – ApA1) is used in many studies in order to get an estimate of pro-atherosclerotic LDL-C particles and antiatherosclerotic HDL-C particles. Notably, the ApoB/ApA1 ratio has been shown to be a particular strong predictor of CHD for different age groups, both sexes and also for ethnic groups (Yusuf et al., 2004).

To prevent CVDs, the current available guidelines have prioritized some lifestyle aspects to change these established risk factors: cessation of tobacco use, reduction of salt in the diet and control of blood pressure, consuming fruits and vegetables or healthy food choices, regular physical activity, management of blood lipids and diabetes, weight control and restricting central obesity, and avoiding harmful use of alcohol (Hames, 2014).

1.1.5 Diet and Cardiovascular Disease

For many years now, it has been well known that an unhealthy diet is an important risk factor for CVD. In fact, poor diet together with smoking and poor or no physical activity form the

base for the development of CVDs, as well as the pillar or target for prevention and treatment (Mozaffarian, 2012; Yusuf et al., 2004; Hu and Willett, 2002).

The nutrient that historically has caused most concern with regard to CVD is dietary fat, the culprit in the classic diet-heart hypothesis (Weinberg, 2004). The essence of this hypothesis is that amount of fat in the diet has a negative impact on blood lipids, increasing the level of serum cholesterol, which leads to the development of atheromatous plaques, and then obstructive CHD, ischemia and AMI (Willett and Stampfer, 2013). As a consequence of this campaign, guidelines recommending low fat diets were launched, subsequently increasing the total intake of carbohydrates in populations (Willett and Stampfer, 2013; Weinberg, 2004). Nonetheless, substitution of fat with carbohydrate, especially refined or with high glycemic load, may have contributed to weight gain and obesity, dyslipidemia, diabetes and metabolic syndrome that prevail today (Weinberg, 2004). Based on results from more recent studies, it has been concluded that the types of fat are more imperative for CVD than its amount (Lockheart et al., 2007; Hu et al., 2001). Increasing intake of polyunsaturated fatty acids (PUFA) (Jakobsen et al., 2009), monounsaturated fatty acids (MUFA), fiber, and complex carbohydrates are associated with a healthy cardiovascular status, while refined carbohydrate (Hu et al., 2001; Liu et al., 2000), trans fatty acids (TFA), some types of saturated fatty acids (SFA) (longer-chain saturated FA, i.e., 12:0 – 18:0) and cholesterol have been positively associated with CVD (Hu et al., 2001).

In addition to nutrients, certain food groups have also been recommended for prevention of CVD or to counteract its progression. The food groups that have shown to have a preventive effect on CVD development are: fruit and vegetables, nonhydrogenated plant oils, nuts, whole grains and fish (Lockheart et al., 2007; Hu and Willett, 2002), and also protein from plants compared to animal proteins (Chalvon-Demersay et al., 2016).

Many dietary guidelines have made their recommendations to combat CVDs based on the Mediterranean diet (Hames, 2014). Those recommendations comprehend reducing SFA intake, TFA and cholesterol, to include omega-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid) in the diet, limiting salt intake, eating plant sources of stanols and sterols to reduce cholesterol, to consume fruits and vegetables daily, include nuts in the diet and fiber, substitute some animal protein by soya protein, refined carbohydrates are to be avoided, folic acid should exceed 400µg/day, and foods rich in vitamins B₁₂, B₆ and riboflavin should be encouraged too (Hames, 2014). A modest amount of alcohol would have some protective effects on CVDs for those at increased risk (Hames, 2014). Available evidence suggest that the Mediterranean diet can be an effective tool to prevent CVD

1), eggs, liver, peanuts and a variety of meats are especially rich in this nutrient (Blusztajn, 1998). In Europe, the main sources of choline are meat and meat products, milk and dairy, grain and its products, egg and egg products, composite dishes and fish and seafood for all age groups (Vennemann et al., 2015).

Table 1. Total choline and choline species content in different foods (mg/ 100 g)

Food item	TC	FC	GPC	Pcho	Ptdcho	SM
Beef liver, cooked, pan fried	420	57	78	12	250	24
Egg, whole, cooked, hard boiled	230	0.7	0.5	0.5	210	14
Soybean, mature seeds, raw	120	47	2.9	1.1	65	0
Pistachio nuts, dry roasted, with salt added	71	11	1.7	8.5	51	0
Fish, salmon, sockeye, cooked, dry heat	66	8.6	5.9	1,1	48	1.8
Peanuts, all types, raw	53	18	1.3	1.8	32	0
Cereals ready-to-eat, Kellogg's ALL-BRAN Original	49	26	4.3	1.7	18	0
Bread, whole-wheat, commercially prepared	27	18	4.9	0.3	3.3	0
Potato, white, flesh and skin, baked	14	6.8	2.7	0.9	4.1	0
Milk, 1% milkfat, with added vitamin A	18	4	9.8	1.9	1.2	0.7
Banana, raw	9.8	3.2	5.6	0.5	0.4	0
Spinach, frozen, chopped, cooked, boiled, drained, without salt	9.4	0.5	0.7	2.4	5.7	0
Rice, brown, long-grain. Cooked	9.2	4.7	1.2	0	3.4	0
Orange, raw, navel	8.4	4.7	1.1	0.5	2.1	0
Spaghetti, cooked, enriched, without added salt	6.4	3.5	0.8	0	2.2	0

FC: Free choline; GP: Glycerophosphocholine; PC: Phosphocholine; Ptdcho: Phosphatidylcholine; SM: Sphingomyelin; TC: Total choline

Source: USDA Database, 2008, Release Two.

In 1998, the Food and Nutrition Board of the Institute of Medicine (IOM) published recommendations for adequate intakes (AI) for choline. At that time, there was not enough data to establish the Estimated Adequate Requirement (IOM, 1998). The recommendations were based on reported association between low dietary choline intake and liver damage (IOM, 1998). For other age groups than adults, the AI values were extrapolated from the AIs for adults. The IOM has also given tolerable upper intake levels (UL). The UL is 3.5g/ d of choline after observation of hypotension at an ingestion of 7.5g/ d. A very high intake of choline can cause hypotension, sweating, diarrhea, fishy body odor (IOM, 1998; Li and Vance, 2008), and vomiting (Li and Vance, 2008).

In 2016, the European Food Safety Authority (EFSA) established AIs based on average intake of choline by healthy adults in nine countries in the EU in an assessment done by Vennemann and colleagues, 2015 (EFSA, 2016). In addition to the average choline intake in nine EU countries, EFSA also considered some studies that showed that depleted individuals who presented organ dysfunction, in general, needed an intake of around 400 mg of choline/ 70 kg of body weight per day to become replete (da Costa et al., 2014; da Costa et al., 2011; Spencer et al., 2011; Fischer et al., 2010; Sha et al., 2010; Fischer et al., 2007;

Niculescu et al., 2007; da Costa et al., 2006; da Costa et al., 2005; Kohlmeier et al., 2005; Zeisel et al., 1991). Alike IOM, in the lack of proper data from younger groups, the EFSA established AI for some age groups through extrapolation from adult's needs. To estimate the AI for children, body weight and growth factors were accounted for. These estimated values are somewhat lower than the AIs given by IOM (Table 2).

Table 2. Choline intake recommendations by IOM, 1998 and EFSA, 2016

IOM	AI (mg/d)		UL	EFSA	AI (mg/d)
	Male	Female			
≥ 19 y	550	425	3.5	All adults	400
14 – 18y	500	400	3.0	15 – 17y	400
9 – 13y	375	375	2.0		
4 – 8y	250	250	1.0		
1 – 3y	200	200	1.0	1 – 3y	140
6 – 12 m	150	150	-	7 – 11 m	160
0 – 6 m	125	125	-		
Pregnant	-	450	-	Pregnant	480
Lactating	-	550	-	Lactating	520

Adequate intake; UL: Upper level of intake

Like most other nutrients, choline requirement seems to be influenced by gestation, lactation (as breastmilk is a source of choline for the infant), stage of development, and sex (EFSA, 2006; IOM, 1998). Requirements for choline in men, premenopausal and postmenopausal women have been evaluated and the choline requirements varied greatly between individuals (Fischer et al., 2007; Kohlmeier et al., 2005). Subjects showed deficiency at different levels of choline supply, and required different amounts of it to replete. Furthermore, it took less or more time for the different individuals to become choline depleted. Both studies reported also that premenopausal women were more resistant to choline deficiency than men and postmenopausal women (Fischer et al., 2007; Kohlmeier et al., 2005). Estrogen seems to promote the activity of one of the enzymes involved in the endogenous synthesis of choline (Zeisel and Corbin, 2012). So, endogenous production of choline alone does not cover the biological needs for choline of human beings (Rajaie and Esmailzadeh, 2011; Cho et al., 2006), but estrogen may decrease the dietary requirements of choline in women (Zeisel and Corbin, 2012).

Additionally, an unknown number of individuals present one or more single nucleotide polymorphisms (SNP), a mutation in one or more genes involved in choline and folate metabolism that alter dietary choline requirements in men and women (Zeisel and Corbin, 2012; Zeisel, 2011; Kohlmeier et al., 2005). Current recommendation of choline intake may change when knowledge of genetic variations is better understood (Zeisel, 2012; Zeisel and da Costa, 2009).

In almost all men and postmenopausal women (Zeisel, 2013), a very low intake of choline (< 50 mg/d) is associated with muscle damage and fatty liver, and further liver damage (Zeisel et al., 1991; Zeisel and Corbin, 2012) with release of liver enzymes into the blood (Fischer et al, 2007). Deficiency of choline cause hepatic steatosis in individuals receiving total parenteral nutrition (Buchman et al., 2001). However, choline deficiency due to very low intake is rare in healthy populations (Cho et al, 2006; Fischer et al., 2005; Buchman et al., 2001).

1.2.2 Digestion, Absorption and Transport of Choline

Choline is rapidly absorbed in the intestines via transporters or carriers depending on choline concentration gradient and on the electrical potential of the membrane of enterocytes, and on the capacity of the transporters (EFSA, 2016). Hydrosoluble choline forms – free choline, phosphocholine and glycerophosphocholine – enter the portal circulation of the liver after digestion by pancreatic and mucosal enzymes (Zeisel and Corbin, 2012), while liposoluble forms – phosphatidylcholine and sphingomyelin – will be hydrolyzed by phospholipases or incorporated by chylomicrons and enter the lymph and distributed to liver and other organs (Zeisel and Corbin, 2012; McDowell, 2008). Free choline is transported in the aqueous phase of plasma, while phosphatidylcholine, phosphocholine, glycerophosphocholine and sphingomyelin are bound to lipoproteins. Dietary phosphatidylcholine and glycerophosphocholine appear in plasma mainly as free choline (EFSA, 2016).

Choline is mainly depleted via oxidation or excretion of phosphatidylcholine in bile (Li and Vance, 2008).

1.2.3 Metabolism

1.2.3.1 Choline Metabolism

Choline is supplied via the diet and is also endogenously produced from phosphatidylethanolamine via phosphatidylethanolamine-N-transferase (PEMT) (Li and Vance, 2008) primarily in the liver (Zeisel and Corbin, 2012). The phosphatidylcholine resulting from the *de novo* pathway can, then, generate choline by action of phospholipases (Li and Vance, 2008).

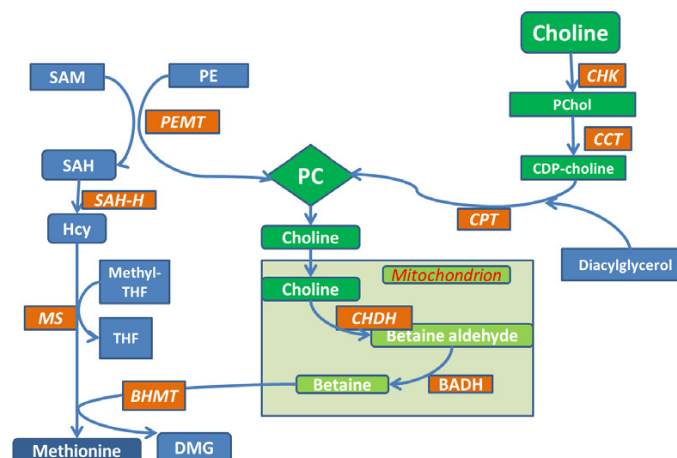


Figure 2: Choline Metabolism and synthesis of phosphatidylcholine via the CDP-choline pathway and PEMT. Left shows the endogenous synthesis of Phosphatidylcholine (PC) (PEMT pathway); right the synthesis of PC from (dietary) choline (CDP pathway). BADH: betaine aldehyde dehydrogenase; BHMT: betaine homocysteine methyltransferase; CCT: phosphocholine cytidyltransferase; CDP-choline, cytidine diphosphocholine; CHK: choline kinase; CHDH: choline oxidase (or dehydrogenase); CPT: CDP-choline diacylglycerol choline phosphotransferase; DMG: dimethylglycine; Hcy: homocysteine; methyl-THF: methyltetrahydrofolate; MS: methionine synthase; PChol: phosphocholine; PE: phosphatidylethanolamine; PEMT: phosphatidylethanolamine N-methyltransferase; PC: phosphatidylcholine; SAH: S-adenosylhomocysteine; SAH-H: S-adenosylhomocysteine hydrolase; SAM: S-adenosylmethionine; THF: tetrahydrofolate. Source: EFSA, 2016.

Synthesis of phosphatidylcholine occurs via cytidine diphosphocholine pathway (CDP-choline) (Obeid, 2013; DeLong et al., 1999), which is one of the two branches of the Kennedy Pathway. This pathway is ubiquitous and present in all body cells (Zeisel and Corbin, 2012). But, in hepatic cells, CDP-choline is responsible for 70% of phosphatidylcholine synthesis, while the other 30% result from the PEMT pathway (Obeid, 2013; Zeisel and da Costa, 2009). (Figure 2).

1.2.3.2 Choline Oxidation

Choline has a major role in the methionine (MET) cycle, which is crucial for normal growth and development (Hollenbeck, 2010). In this process, MET, an essential amino acid (Brustolin et al. 2010), is recycled in the cell when homocysteine (Hcy) receives a methyl-

group from choline (via betaine) or from 5-methyltetrahydrofolate (5-MTHF) (Obeid, 2013; Blom and Smulders, 2011; Brustolin et al., 2010). At this point, i.e. where Hcy is converted to MET, the metabolisms of choline (betaine), folate and MET are interconnected (Niculescu and Zeisel, 2002; Zeisel and Blusztajn, 1994). Abnormalities in this cycle have been associated with CVD (Ueland et al., 2000) among, other diseases (Hollenbeck, 2010) as, for example, hepatosteatosis (Mato et al., 2008).

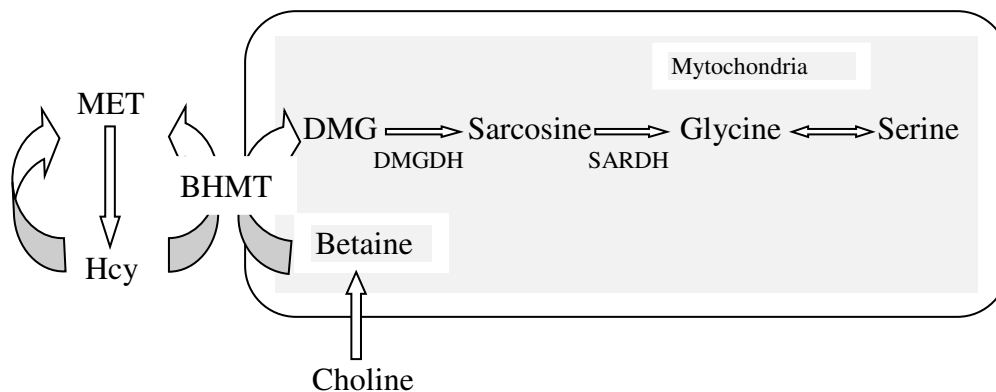


Figure 3. Choline Oxidation Pathway. BHMT: Betaine homocysteine methyltransferase; DMG: Dimethylglycine; DMGDH: Dimethylglycine dehydrogenase; Hcy: Homocysteine; MET: Methionine; SARDH: Sarcosine dehydrogenase

Before choline can remethylate Hcy, it needs to be metabolized to betaine (also known as trimethylglycine), via action of the enzyme betaine-aldehyde. Betaine, from diet or from choline, donates a methyl group to Hcy, via betaine homocysteine methyltransferase (BHMT), turning it into MET. In this process, dimethylglycine (DMG) is produced (Obeid, 2013). DMG can be eliminated in the urine in small quantities, and most of it will be dehydrogenated producing sarcosine (Obeid, 2013). When sarcosine is produced, a methyl-group is donated to tetrahydrofolate, regenerating 5-MTHF. Sarcosine will make glycine by action of sarcosine dehydrogenase.

Choline is also important in the production of S-adenosylmethionine (SAM) (Figure 2). As a universal methyl donor, SAM is of major importance for numerous reactions, including genetic and epigenetic regulation through methylation (Obeid, 2013). SAM results of the transfer of an adenosyl molecule to MET by a tissue specific methionine adenosyltransferase (Obeid, 2013; Blom and Smulders, 2011; Halsted et al., 2002).

Accumulation of SAM decreases the use of betaine and of 5-MTHF (Obeid, 2013; Halsted et al., 2002), as sources of methyl-group (Obeid, 2013). In addition, high concentration of SAM activates the initial enzyme of the transsulphuration pathway,

cystathionine β -synthase, which requires vitamin B6 as cofactor (Blom and Smulders, 2011). This pathway irreversibly removes Hcy from the cell (Obeid, 2013) and produces cysteine, which will be used in other reactions.

When SAM donates a methyl-group by action of a methyltransferase (Blom and Smulders, 2011), S-adenosylhomocysteine (SAH), a potent methylation inhibitor (Dong et al., 2002), is formed (Obeid, 2013). SAH is sequentially fragmented into Hcy. In the sequence, low concentrations of SAM allow remethylation of Hcy to happen again (Blom and Smulders, 2011).

Increased levels of SAM trigger the transsulfuration pathway, whereas under low levels of SAM, as in fasting conditions, the remethylation of Hcy is active (Obeid, 2013).

1.2.3.3 Trimethylamine N-Oxide

Choline not absorbed by the enterocytes will be used by the intestinal microbiota (McDowell, 2008). Dietary free choline, betaine (although ~100 times less efficiently than choline) (Wang et al., 2014; Wang et al., 2011), carnitine (Koeth et al., 2013), and phosphatidylcholine (Tang et al., 2013) are metabolized in the gut to trimethylamine (TMA). The production of TMA is dependent on interindividual variations of gut microflora composition (Tang and Hazen, 2014; Wang et al., 2014; Koeth et al., 2013; Wang et al., 2011). TMA is, then, converted into trimethylamine N-oxide (TMAO) in the liver by a family of flavin monooxygenase enzymes called FMO, of which flavin monooxygenase 3 (FMO3) seems to be the most relevant for TMAO synthesis. The hepatic FMO3 genotype of an individual is another determinant factor for TMAO production (Cho et al., 2016). Individuals presenting a defect on FMO3 present trimethylaminuria (fish malodor syndrome), which is the accumulation of the gas TMA that smells like rotting fish (Wang et al., 2011).

TMAO functions in human beings remain uncertain so far (Wang et al., 2011). Nonetheless, high plasma levels of TMAO have been associated with AMI (Wang et al., 2011) and with cardiometabolic disorders (Tang and Hazen, 2014; Koeth et al., 2013) that need to be explored further.

1.2.4 Biological Functions of Choline

The major fates of choline are to donate methyl groups via betaine (Corbin and Zeisel, 2012) and to produce phosphatidylcholine (Corbin and Zeisel, 2012; Zeisel and Corbin, 2012; Gibellini and Smith, 2010; Li and Vance, 2008). Phosphatidylcholine is the most abundant

(95%) choline-containing molecule in mammalian tissues (Ueland, 2011). Choline phospholipids contribute to structural integrity (Zeisel et al., 1991) and signaling functions of cell membranes (Zeisel and Canty, 1993; Zeisel and Corbin, 2012), as 1,2-sn-diacylglycerol, sphingosine, and ceramide, which are three important intracellular messengers (Zeisel and Canty, 1993).

Choline is essential for hepatic lipid homeostasis (Zeisel and Corbin, 2012; Vance et al., 2007). Packaging and transportation of TG in the liver are dependent on the supply of phosphatidylcholine (Yao and Vance, 1988), via hepatic PEMT, for the formation of VLDL-C (Yao and Vance, 1988; Zeisel et al., 1991), in a way that other phospholipids cannot substitute (Yao and Vance, 1988; Zeisel et al., 1991). Choline phospholipids are also constituents of bile (Tang and Hazen, 2014).

Choline affect the concentration of SAM through its capacity of donating methyl-groups via its metabolite betaine (EFSA, 2016). SAM in altered concentrations may modify DNA methylation, and then influence gene transcription, genomic imprinting, and genomic stability (Ueland, 2011). Choline is also a nutrient of evidenced importance for the formation of the human brain (Zeisel and Corbin, 2012).

Betaine serves as an osmolyte (Obeid, 2013; Craig, 2004) in the kidney to support water reabsorption (Zeisel and Corbin, 2012). In addition, betaine works also stabilizing the structure of proteins in denaturing conditions and cell volume (Obeid, 2013). Betaine has an important methionine-sparing effect, making MET more available for protein synthesis, and it spares choline as well, which can be used for lipid metabolism (Obeid, 2013).

1.2.5 Choline Intake and Acute Myocardial Infarction

Considering the importance of choline, dietary intake of choline (and betaine) has been assessed in some epidemiological studies together with important CVD risk factors. Choline intake has predicted plasma total homocysteine (tHcy) concentrations (Cho et al., 2006). And through a number of different mechanisms choline has been positively linked with increased risk of CVD mortality (Zheng et al., 2016), negatively linked (Millard et al., 2016), and not linked with CVD risk (Nagata et al., 2015; Dalmeijer et al., 2008; Bidulescu et al., 2007). There is, therefore, contradictory evidence around choline effects on cardiovascular health, and more studies are necessary to elucidate this topic.

2. Aim of the Study

The aim of this project is to investigate the association between dietary choline intake and risk of AMI in patients with established SAP.

The null-hypothesis is that choline intake is not associated with the subsequent risk of AMI in these patients.

3. Methods

3.1 Study Population and Design

The current study population is a subpopulation from the prospective, randomized, double-blind controlled trial The Western Norway B Vitamin Intervention Trial (WENBIT), with a total of 3090 participants, conducted in two university hospitals in Bergen and Stavanger between 1999 and 2005 (main recruitment period between 2000 and 2004) (Ebbing et al., 2008). The WENBIT trial was terminated in 2005 and the mean follow-up of intervention was four years. For the original WENBIT population the exclusion criteria were unavailability for follow-up, participation in other trials, known alcohol abuse, serious mental illness, or cancer. Dietary data were collected at baseline by using a food frequency questionnaire (FFQ). For the current study exclusion criteria were not answered FFQ (n = 606) or FFQ with more than 1 blank page (n = 96), reported energy intake under 3000 kJ or 3300 kJ for women and men, respectively, or above 15000 kJ or 17500 for women and men, respectively (n = 37), and a diagnose of acute coronary syndrome (ACS) at baseline (n = 332), which left 2019 individuals for the current study (Figure 3). Mean follow-up time for this study was 7.2 (2.4) years.

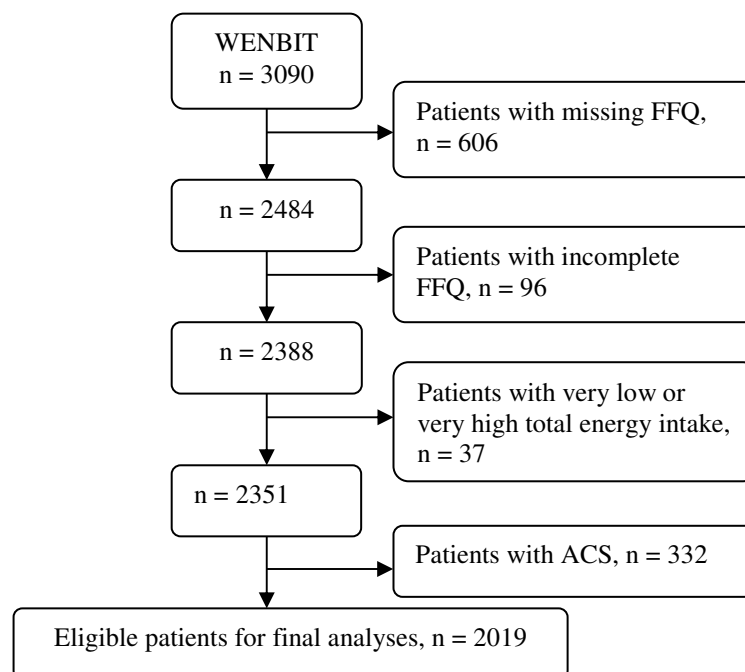


Figure 4. Flowchart over study population

3.2 Ethical Statement

Written informed consent was obtained from the subjects on the day of randomization. The study protocol was in accordance with the principles of the Declaration of Helsinki and the trial was approved by the Regional Committee for Medical and Health Research Ethics, the Norwegian Medicines Agency, and the Data Inspectorate (Ebbing et al., 2008).

3.3 Baseline Characteristics

Demographic and clinical data were obtained at baseline. Anthropometrical measurement such as weight and height were obtained, and BMI was calculated by weight in kilograms divided by the square of the height in meter. Participants were defined as current smokers based on self-reported smoking habits or on serum cotinine (predominant metabolite of nicotine). Individuals with serum cotinine ≥ 85 nmol/L were included in the definition of current smokers regardless of their self-report on smoking. Diabetes mellitus, including both types 1 and 2, was defined by preexisting diagnosis, and hypertension was defined according to current use of antihypertensive medications. Left ventricular ejection fraction (LVEF) was obtained either by ecocardiography or by ventriculography performed during cardiac catheterization. The extent of coronary artery disease (CAD) was scored by aggregating the number of significantly stenotic arteries (significant stenosis was defined by luminal narrowing $\geq 50\%$ of any epicardial coronary artery) to a maximum of three.

3.4 Laboratory Analyses

Blood samples were collected at baseline. Some patients fasted before the blood samples were collected, and some others did not. Plasma choline, betaine, tHcy, TMAO, DMG, and serum cotinine, blood lipids, glucose and C-reactive protein (CRP) were analyzed. Serum lipids and glucose were measured using fresh samples at the hospital laboratories at Stavanger University Hospital, Stavanger, or Haukeland University Hospital, Bergen, Norway. Cotinine, choline, TMAO, betaine and DMG were measured using gas chromatography coupled to tandem mass spectrometry, while tHcy was measured using matrix assisted laser desorption ionization-time of flight mass spectrometry. Cotinine was measured using liquid chromatography combined with mass spectrometry. The measurements were performed at Bevital AS, Bergen, Norway. Estimated glomerular filtration rates (eGFR)

were obtained using the Chronic Kidney Disease Epidemiology Collaboration equation (Levey et al., 2009).

3.5 Dietary Assessment

At baseline, the study participants were asked to fill out a FFQ (Appendix) developed at the Department of Nutrition, Institute of Basic Medical Sciences of the University of Oslo in the Norwegian language. The FFQ was self-administered and it was returned by mail or at the one-month follow-up appointment. On the first page a short instruction on how to answer the FFQ was presented. The answers were read through optical mark reading (Nes et al., 1992).

The applied FFQ was an adaptation from a 180-item FFQ, designed in 1992, which targeted to assess the habitual food intake of Norwegian adults and intended for use in epidemiological studies of diet and health. The adaptation resulted in a 169-item FFQ with the purpose to measure the average diet over the past year. It contained daily meals and their frequency of intake. The dietary pattern was according to the Norwegian dietetic habits where bread-based meals are important. Questions on the use of vitamin and mineral supplements were included. There were no questions on choline supplement. The portion sizes were assessed using household measures (such as slices, glasses, cups, pieces, spoons), units (dl, hg or g) for each food. Frequency of ingestion was possible for the period of a day, week, or a month depending on the food item or never consumed (Nes et al, 1992).

For estimation of nutrient and food intake, a software system developed at the Department of Nutrition, University of Oslo (Kostberegningssystem, version 3.2) was used. The food database is mainly based on the official Norwegian food composition table (National Nutrition Council, 1995), with some additional foods.

Intake of choline and the individual choline species and betaine was quantified using the U.S. Department of Agriculture (USDA) Database for the Choline Content of Common Foods, release 2 (Patterson et al., 2008). The total dietary intake of choline was estimated as the sum of free choline, phosphatidylcholine, phosphocholine, glycerophosphocholine and sphingomyelin. For food items that occurred in both the current FFQ and in the USDA database, the available contents of choline and betaine were used. For food items in the current FFQ not corresponding to the ones found in the USDA database, choline and betaine contents were estimated using nutritionally equivalent foods. For dishes or items which differentiated from the ones in the USDA database, contents of choline and betaine were calculated for each ingredient in the FFQ recipe.

Alcohol intake was also used in our analysis. According to the Nordic Nutrition Recommendations (NNR, 2012), the consumption of alcohol should not exceed 10 g per day for women and no more than 20 g per day for men. Partly based on this, alcohol consumption was divided into four categories: no intake (0 g of alcohol); low-moderate (under 10 g of alcohol per day for women and 20 g per day for men); moderate (between 10 and 20 g of alcohol per day for women and between 20 and 30 g of alcohol per day for men); and high-moderate (above 20 g of alcohol per day for women and 30 g of alcohol per day for men).

3.6 Clinical End Points

The primary end point of the current study was incident AMI, included fatal and nonfatal events and were defined according to the International Classification on Diseases (ICD) 10th edition, I21-22. Information on endpoints was obtained from the Cardiovascular Disease in Norway project (CVDNOR, <http://cvdnor.b.uib.no/>), which provided information on discharge diagnoses from most Norwegian public hospitals and from the Cause of Death during 1994 – 2009, and linked to each patient's unique 11-digit personal number.

3.7 Statistical Analyses

Because the effect of nutrients may be confounded by total energy intake, total intake of choline and choline species was adjusted for total energy intake by using the residual method (Willett et al., 1997). Energy-giving components and foods were adjusted using the nutrient density method and presented as percent of total energy intake (carbohydrate, protein, fat, and alcohol) or as g per 1000 kcal (fiber, vegetables, fruits and berries).

Baseline characteristics by quartiles of total choline intake and by incident AMI are presented. Continuous variables are presented as means (SD) and categorical variables as counts (%). Linear trend (p for trend) was estimated using linear regression for continuous variables and logistic regression for categorical variables. Fisher's exact test for multicategorical variables (for alcohol specifically Pearson's chi-square test was used). The calculated p values are 2-sided and considered statistical significant if less than 0.05.

For estimating the hazard risk of experiencing an AMI during the study period, Cox proportional hazards regression model was used. Hazard ratios were calculated for each 100 mg raise of total choline intake and, in sequence, for each 10 mg raise of free choline, phosphatidylcholine, glycerophosphocholine, phosphocholine and sphingomyelin intake.

Model 1 was adjusted for total caloric intake. Model 2 was also adjusted for sex, age, smoking, previous AMI, previous coronary artery bypass grafting (CABG) and extension of CAD at baseline. Intervention allocation group was added to model 2, but it did not materially alter the results so it was not included in the model. Moreover, adjustment for intake of SFA, carbohydrate, fiber, protein, alcohol consumption, intake of vegetables, fruits and berries, plasma TMAO, use of aspirin and serum lipids did not affect the model materially and was not included in the final model. Model 3 was similar to model 2 plus adjustment for BMI and diabetes.

To explore potential non-linear relationships between choline intake and incidence of AMI a general additive model (GAM) was plotted.

For statistical analyses, IBM SPSS Statistics versions 23 and 24 for Windows were used. For GAM, R version 3.3.1 (The R Foundation for Statistical Computing, Vienna, Austria) was used. The calculated p-values are 2-sided and considered statistical if less than 0.05.

4. Results

4.1 Baseline characteristics

The mean follow-up time was 7.2 (2.4) years. Baseline characteristics of the study population across quartiles of total choline intake are presented in table 3.

Table 3. Baseline characteristics in 2019 patients with stable angina pectoris by quartiles of total choline intake

	Total	Q1 n = 504	Q2 n = 505	Q3 n = 505	Q4 n = 505	<i>P</i> _{trend}
Total choline intake, mg/d	294 (65.1)	223 (27.7)	272 (9.58)	306 (10.8)	377 (58.4)	
Men, n (%)	1610 (79.7)	432 (85.7)	381 (75.4)	388 (76.8)	409 (81.0)	0.11
Age, y	61.8 (9.72)	61.4 (10.3)	62.6 (9.29)	62.2 (9.99)	61.0 (9.24)	0.41
BMI, kg/m ²	26.3 (3.73)	25.8 (3.71)	26.2 (3.76)	26.3 (3.64)	27.0 (3.75)	< 0.001
Current smokers, n (%)	593 (29.4)	132 (26.2)	136 (26.9)	143 (28.3)	182 (36.0)	0.001
Hypertension, n (%)	958 (47.4)	220 (43.7)	240 (47.5)	240 (47.5)	258 (51.1)	0.025
Diabetes mellitus, n (%)	226 (11.2)	38 (7.5)	48 (9.5)	55 (10.9)	85 (16.8)	< 0.001
LVEF, %	63.9 (11.1)	64.4 (10.5)	64.1 (11.7)	63.8 (10.9)	63.3 (11.5)	0.09
Previous MI n (%)	867 (42.9)	209 (41.5)	216 (42.8)	207 (41.0)	235 (46.5)	0.17
Previous CABG n (%)	290 (14.4)	74 (14.7)	71 (14.1)	67 (13.3)	78 (15.4)	0.83
Previous PCI n (%)	452 (22.4)	129 (25.6)	94 (18.6)	112 (22.2)	117 (23.2)	0.66
Extent of CAD n (%)						< 0.001
No stenotic vessels ^c	248 (12.3)	70 (13.9)	50 (9.9)	66 (13.1)	62 (12.3)	
1-vessel disease ^c	568 (28.2)	130 (25.8)	163 (32.3)	129 (25.5)	146 (28.9)	
2-vessel disease ^c	549 (27.2)	141 (28.0)	127 (25.1)	143 (28.3)	138 (27.3)	
3-vessel disease ^c	653 (32.4)	162 (32.2)	165 (32.7)	167 (33.1)	159 (31.5)	
Serum glucose, mmol/L	6.28 (2.14)	6.14 (2.03)	6.09 (1.89)	6.25 (2.04)	6.64 (2.52)	< 0.001
S-CRP, mg/L	3.26 (6.26)	3.18 (4.99)	3.38 (8.04)	3.21 (5.95)	3.26 (5.67)	0.96
eGFR, mL/min/1.73m ² ^a	89.7 (15.4)	90.6 (14.9)	88.4 (14.9)	89.1 (16.2)	90.8 (15.4)	0.70
Plasma levels of 1-carbon metabolites						
Choline, μmol/L ^a	9.84 (2.53)	9.88 (2.39)	9.76 (2.40)	9.86 (2.62)	9.88 (2.69)	0.83
TMAO, μmol/L ^d	8.70 (9.94)	7.87 (8.44)	7.79 (8.40)	9.22 (10.9)	9.92 (11.5)	< 0.001
Betaine, μmol/L ^a	41.0 (13.0)	42.1 (13.6)	39.9 (12.3)	41.5 (12.9)	40.4 (13.1)	0.17
DMG, μmol/L ^a	4.26 (1.64)	4.36 (1.68)	4.15 (1.49)	4.43 (1.99)	4.10 (1.31)	0.13
tHcy, μmol/L ^a	10.9 (3.83)	11.3 (3.80)	11.0 (4.20)	10.9 (3.98)	10.4 (3.25)	< 0.001
Serum Lipids and Apolipoproteins						
TC, mmol/L ^c	5.05 (1.19)	5.01 (1.13)	5.05 (1.09)	5.07 (1.44)	5.09 (1.10)	0.27
LDL-C, mmol/L ^b	3.06 (1.02)	3.06 (1.01)	3.05 (0.97)	3.06 (1.08)	3.09 (1.02)	0.62
HDL-C, mmol/L ^c	1.27 (0.35)	1.25 (0.34)	1.29 (0.36)	1.28 (0.35)	1.26 (0.34)	0.60
TG, mmol/L ^c	1.79 (1.15)	1.77 (0.92)	1.76 (0.94)	1.77 (1.53)	1.85 (1.09)	0.30
ApoB-100, g/L	0.88 (0.23)	0.88 (0.23)	0.88 (0.23)	0.88 (0.27)	0.89 (0.23)	0.29
ApoA1, g/L	1.28 (0.25)	1.28 (0.25)	1.28 (0.25)	1.28 (0.26)	1.28 (0.24)	0.95
ApoB-100/ApoA1	0.71 (0.23)	0.71 (0.24)	0.71 (0.23)	0.71 (0.25)	0.72 (0.23)	0.48
LDL-C/ApoB ^b	3.47 (0.65)	3.49 (0.72)	3.47 (0.59)	3.48 (0.60)	3.45 (0.67)	0.4
HDL-C/ApoA1 ^c	0.99 (0.19)	0.97 (0.17)	1.00 (0.20)	1.00 (0.21)	0.99 (0.18)	0.26
Medications, n (%)						
β-Blocker	1547 (76.6)	393 (78.0)	381 (75.4)	390 (77.2)	383 (75.8)	0.58
ACEI and/or ARB ^c	630 (31.2)	130 (25.8)	157 (31.1)	166 (32.9)	177 (35.0)	0.001
Statin ^c	1781(88.3)	437 (86.9)	440 (87.1)	452 (89.5)	452 (89.5)	0.11
Aspirin	1801 (89.2)	464 (92.1)	451 (89.3)	439 (86.9)	447 (88.5)	0.036

^a n = 2016;

^b n = 2017;

^c n = 2018;

^d n = 2006. All other variables have a n = 2019

Variables are reported as mean (SD) unless otherwise noted. Diabetes mellitus include both type 1 and type 2. ACEI, angiotensin-converting enzyme inhibitor and ARB, angiotensin receptor blocker; ApoB-100/ApoA1, ApoB-100/ApoA1 ratio; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CRP, C-reactive protein; DMG, dimethylglycine; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; HDL-C/ApoA1, HDL-C/ApoA1 ratio; LDL-C, low-density lipoprotein cholesterol; LDL-C/ApoB, LDL-C/ApoB ratio; LVEF, left ventricular ejection fraction; AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; TC, total cholesterol; TG, triglycerides; tHcy, total homocysteine; TMAO, trimethylamine N-oxide. P for trend was estimated using linear regression analysis for continuous variables, logistic regression for dichotomous variables and p value was estimated using Fisher's exact test, for multicategorical variables.

There was a statistically significant positive association between increasing choline intake and BMI ($p < 0.001$), hypertension ($p = 0.025$), diabetes ($p < 0.001$), smoking ($p = 0.001$), serum glucose ($p < 0.001$), use of ACEI/ARB (angiotensin-converting enzyme inhibitor and angiotensin receptor blocker) ($p = 0.001$) and aspirin medications ($p = 0.036$). Higher intake of total energy-adjusted choline was inversely associated with plasma tHcy ($p < 0.001$), and positively associated with plasma TMAO ($p < 0.001$). It was not observed any association between total choline intake and age, gender, extent of CAD, LVEF, previous AMI, previous coronary intervention, CRP, eGFR, serum lipids and apolipoproteins, plasma choline, plasma betaine, plasma DMG or use of β -blocker and statin (Table 3).

4.2 Dietary Intake

Dietary intake by quartiles of total choline intake is shown in table 4. The mean total energy intake (SD) was 2095 (633) kcal/d, the mean total energy-adjusted choline intake was 294 (65.1) mg/d. Forty-three percent of the total choline came from phosphatidylcholine, 127 (36.9) mg/d, followed by free choline, 75.6 (17.3) mg/d. Mean energy-adjusted choline intake for quartiles 1, 2, 3 and 4 was 223 (27.7) mg/d, 272 (9.58) mg/d, 306 (10.8) mg/d, 377 (58.4) mg/d.

Table 4. Daily dietary intake by quartiles of total choline intake

	Total n = 2019	Q1 n = 504	Q2 n = 505	Q3 n = 505	Q4 n = 505	P _{trend}
Total choline, mg	294 (65.1)	223 (27.7)	272 (9.57)	305 (10.8)	377 (58.3)	
Energy, kcal	2095 (633)	2236 (637)	1962 (607)	2020 (614)	2163 (638)	0.21
Carbohydrate, E%	49.7 (6.38)	51.2 (6.42)	50.7 (5.53)	49.5 (6.29)	47.4 (6.56)	<0.001
Fiber, g/1000 kcal	12.2 (3.21)	11.5 (2.74)	12.3 (2.78)	12.3 (3.08)	12.6 (3.99)	<0.001
Protein, E%	16.9 (2.56)	15.3 (2.18)	16.6 (2.12)	17.2 (2.14)	18.6 (2.60)	<0.001
Fat, E%	31.3 (5.41)	31.8 (5.47)	30.9 (5.06)	31.3 (5.57)	31.2 (5.49)	0.20
SFA, E%	11.7 (2.61)	12.2 (2.72)	11.7 (2.58)	11.6 (2.64)	11.4 (2.45)	<0.001
MUFA, E%	10.3 (1.96)	10.3 (1.99)	10.1 (1.86)	10.3 (1.99)	10.3 (2.00)	0.43
PUFA, E%	7.19 (1.96)	7.33 (2.05)	6.96 (1.84)	7.22 (1.94)	7.27 (1.98)	0.79
Alcohol, E%	2.00 (3.08)	1.62 (2.72)	1.76 (3.16)	1.93 (2.39)	2.70 (3.76)	<0.001
Alcohol intake, n (%) ¹						< 0.001
No intake	508 (25.2)	164 (32.5)	146 (28.9)	112 (22.2)	86 (17.0)	
Low-moderate	1352 (67.0)	307 (60.9)	333 (65.9)	362 (71.7)	350 (69.3)	
Moderate	93 (4.6)	19 (3.8)	16 (3.2)	19 (3.8)	39 (7.7)	
High	66 (3.3)	14 (2.8)	10 (2.0)	12 (2.4)	30 (5.9)	
Betaine, mg	139 (38.0)	141 (39.5)	137 (32.5)	140 (39.1)	136 (40.3)	0.16
Free choline, mg	75.6 (17.3)	62.3 (11.1)	71.5 (10.3)	76.9 (11.8)	91.6 (19.3)	<0.001
Phosphatidylcholine, mg	127 (36.9)	96.4 (21.2)	117 (20.3)	133 (25.5)	162 (40.6)	<0.001
Sphingomyelin, mg	9.49 (4.04)	6.27 (2.38)	8.50 (2.01)	10.1 (2.59)	13.0 (4.96)	<0.001
Phosphocholine, mg	11.5 (5.34)	7.65 (3.18)	10.2 (3.06)	12.2 (3.87)	16.1 (6.43)	<0.001
Glycerophosphocholine, mg	62.5 (26.7)	42.3 (15.4)	56.9 (14.8)	65.6 (18.2)	85.9 (32.8)	<0.001
Fruit and berries, g/ 1000 kcal [*]	125 (85.3)	113 (85.1)	128 (80.8)	130 (86.9)	128 (87.3)	0.004
Vegetables, g/ 1000 kcal	105 (74.1)	75.2 (43.3)	95.6 (52.5)	111 (66.6)	140 (103)	<0.001

Variables are reported as mean (SD) unless otherwise noted.

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

¹No intake: 0 g/day; Low-moderate: women 0.1-10 g/day, men 0.1-20 g/day; Moderate: women 10-20 g/d, men 20-30 g/d; High: women >20 g/day, men >30 g/day.

Choline and betaine intake was adjusted for total energy intake using the residual method. Intake of macronutrients and foods were adjusted for total energy intake using the nutrient density method presented as either E% or g/1000 kcal.

^{*}Fruit includes canned and fresh fruits as well as juice.

P for trend was estimated using linear regression analysis for continuous variables and Fisher's exact test for categorical variables.

Higher intake of energy-adjusted choline was inversely associated with intake of carbohydrates ($p < 0.001$), SFA ($p < 0.001$), and positively correlated with intake of fiber ($p < 0.001$), protein ($p < 0.001$), alcohol ($p < 0.001$), fruits and berries ($p < 0.05$) and vegetables ($p < 0.001$) (Table 4). There was no association between intake of betaine and choline.

4.3 Baseline Characteristics by Incidence of Acute Myocardial Infarction

A total of 297 (14.7%) participants experienced an AMI episode. In table 5, baseline characteristics of patients who did or did not experience AMI during the study period are presented. Overall, patients who experienced AMI were older ($p = 0.006$), and a larger proportion of them had already had an AMI episode earlier ($p < 0.001$). They also tended to be sicker compared to the group who did not experience AMI. Risk factors like previous surgeries (CABG, $p < 0.001$, and percutaneous coronary intervention – PCI, $p < 0.05$), extensive CAD (3-vessel CAD, $p = 0.004$), elevated serum glucose ($p = 0.024$), hypertension ($p = 0.024$), diabetes ($p < 0.001$), higher tHcy ($p = 0.036$), higher LDL-C ($p = 0.04$), lower HDL-C ($p = 0.003$), higher ApoB100 ($p = 0.003$), lower ApA1 ($p = 0.039$) and use of ACEI/ARB medication ($p = 0.03$) were more spread among those who had AMI. A higher percentage of people who developed AMI were current smokers ($p = 0.007$).

Table 5. Baseline characteristics by incidence of acute myocardial infarction

	Acute myocardial infarction during follow-up		P
	Yes n = 297	No n = 1722	
Men, n (%)	236 (79.5)	1374 (79.8)	0.88
Age, y	63.4 (10.6)	61.5 (9.54)	0.006
BMI, kg/m ²	26.6 (4.54)	26.3 (3.58)	0.26
Current smokers, n (%)	107 (36.0)	486 (28.2)	0.007
Hypertension, n (%)	159 (53.5)	799 (46.4)	0.02
Diabetes mellitus, n (%)	53 (17.8)	173 (10.0)	<0.001
LVEF, n	60.6 (13.0)	64.4 (10.7)	<0.001
Previous MI, n (%)	180 (60.6)	687 (39.9)	<0.001
Previous CABG, n (%)	69 (23.2)	221 (12.8)	<0.001
Previous PCI, n (%)	84 (28.3)	368 (21.4)	0.01
Extent of CAD, n (%)			0.002
No stenotic vessels ^c	21 (7.1)	227 (13.2)	
1-vessel disease ^c	78 (26.3)	490 (28.5)	
2-vessel disease ^c	80 (26.9)	469 (27.3)	
3-vessel disease ^c	118 (39.7)	535 (31.1)	
Serum glucose mmol/L	6.57 (2.42)	6.23 (2.09)	0.024
S-CRP, (mg/L)	3.55 (4.81)	3.21 (6.48)	0.39
eGFR, mL/min/1.73m ² ^a	87.4 (18.5)	90.1 (14.7)	0.02
Plasma levels of 1-carbon metabolites			
Choline, $\mu\text{mol/L}$ ^a	10.1 (2.70)	9.80 (2.49)	0.057
Betaine, $\mu\text{mol/L}$ ^a	40.4 (11.8)	41.0 (13.2)	0.46
tHcy, $\mu\text{mol/L}$ ^a	11.4 (4.54)	10.8 (3.69)	0.036
TMAO, $\mu\text{mol/L}$ ^e	9.53 (11.4)	8.56 (9.66)	0.17

DMG, $\mu\text{mol/L}^a$	4.42 (1.66)	4.24 (1.64)	0.08
Serum lipids and apolipoproteins			
TC, mmol/L ^c	5.13 (1.24)	5.04 (1.19)	0.21
LDL-C, mmol/L ^b	3.18 (1.11)	3.05 (1.00)	0.04
HDL-C, mmol/L ^c	1.21 (0.32)	1.28 (0.36)	0.003
TG, mmol/L ^c	1.83 (0.93)	1.78 (1.18)	0.47
ApoB-100, g/L	0.92 (0.27)	0.87 (0.23)	0.003
ApA1, g/L	1.25 (0.25)	1.29 (0.25)	0.039
ApoB-100/ApA1	0.76 (0.25)	0.70 (0.23)	<0.001
LDL-C/ApoB ^b	3.44 (0.58)	3.48 (0.66)	0.33
HDL-C/ApA1 ^c	0.97 (0.18)	1.00 (0.20)	0.037
Medications n (%)			
β -blockers	225 (75.8)	1322 (76.8)	0.71
ACEI and/or ARB ^c	109 (36.7)	521 (30.3)	0.03
Statin ^c	254 (85.5)	1527 (88.7)	0.12
Aspirin	255 (85.9)	1546 (89.8)	0.05

^a n = 2016;

^b n = 2017;

^c n = 2018;

^d n = 2006. All other variables have a n = 2019

Variables are reported as mean (standard deviation) unless otherwise noted. Diabetes mellitus include both type 1 and type 2.

ACEI, angiotensin-converting enzyme inhibitor and ARB, angiotensin receptor blocker; ApoB-100/ApA1, ApoB-100/ApA1 ratio; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CRP, C-reactive protein; DMG, dimethylglycine; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; HDL-C/ApA1, HDL-C/ApA1 ratio; LDL-C, low-density lipoprotein cholesterol; LDL-C/ApoB, LDL-C/ApoB ratio; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary

intervention; TC, total cholesterol; TG, triglycerides; tHcy, total homocysteine; TMAO, trimethylamine N-oxide.

P value was calculated using independent samples t-test for continuous variables, and Fisher's Exact test for categorical variables.

4.4 Dietary Intake and Acute Myocardial Infarction Events

With regard to their habitual diet and AMI incidence (Table 6), analyses show that the group of patients who experienced AMI reported a higher intake of total choline 304 (62.8) mg/d vs. 293 (65.4) mg/d ($p = 0.005$). Almost 50% of total choline came from phosphatidylcholine 133 (37.9) mg/d, and phosphatidylcholine intake was higher in the AMI group as well ($p = 0.003$). The AMI patients reported lower total energy intake 2010 (645) kcal/d vs. 2110 (630) kcal/d ($p = 0.012$). More people in the group who experienced an AMI stated no intake of alcohol (31% vs. 24%, $p = 0.014$).

Table 6. Daily dietary intake in groups with or without acute myocardial infarction

	Acute myocardial infarction during follow-up		P
	Yes (n = 297)	No (n = 1722)	
Total choline, mg	304 (62.8)	293 (65.4)	0.005
Energy, kcal	2010 (645)	2110 (630)	0.012
Carbohydrate, E%	49.7 (6.68)	49.7 (6.33)	0.87
Fiber, g/1000 kcal	12.5 (3.42)	12.2 (3.17)	0.16
Protein, E%	17.2 (2.51)	16.9 (2.57)	0.07
Fat, E%	31.2 (5.64)	31.3 (5.37)	0.78
SFA, E%	11.7 (2.77)	11.7 (2.59)	0.85
MUFA, E%	10.3 (2.05)	10.3 (1.95)	0.81
PUFA, E%	7.13 (1.96)	7.21 (1.96)	0.51
Alcohol, E%	1.89 (3.00)	2.02 (3.09)	0.51
Alcohol intake, n (%) ¹			0.09
No intake	92 (31.0)	416 (24.2)	

Low-moderate	183 (61.6)	1169 (67.9)	
Moderate	12 (4.0)	81 (4.7)	
High	10 (3.4)	56 (3.3)	
Betaine, mg	141 (39.1)	138 (37.9)	0.19
Free choline, mg	76.5 (17.6)	75.4 (17.2)	0.32
Phosphatidylcholine, mg	133 (37.9)	126 (36.7)	0.003
Sphingomyelin, mg	10.0 (3.97)	9.39 (4.05)	0.01
Phosphocholine, mg	11.8 (5.31)	11.5 (5.35)	0.31
Glycerophosphocholine, mg	64.7 (26.6)	62.1 (26.8)	0.12
Fruit, g/ 1000 kcal ²	124 (90.2)	125 (84.5)	0.87
Vegetables, g/ 1000 kcal	110 (86.2)	105 (71.8)	0.30

Variables are reported as mean (SD) unless otherwise noted.

GCP, glycerophosphocholine; MUFA, monounsaturated fatty acid; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

¹ No intake: 0 g/day; Low-moderate: women 0.1-10 g/day, men 0.1-20 g/day; Moderate: women 10-20 g/d, men 20-30 g/d; High: women >20 g/day, men >30 g/day.

Choline and betaine intake was adjusted for total energy intake using the residual method. Intake of macronutrients and foods were adjusted for total energy intake using the nutrient density method presented as either E% or g/1000 kcal.

²Fruit includes canned and fresh fruits as well as juice and berries.

P value was estimated using independent samples t-test for continuous variables, and Fisher's exact test, for categorical variables.

4.5 Dietary Choline Intake and Risk of Acute Myocardial Infarction

Three Cox proportional hazards regression models, were created to control for confounding factors on the effect of dietary choline on risk of AMI. In the crude model, adjusting only for energy intake, the risk of AMI increased with 28% (CI 9 - 49) for each 100 mg increase in total choline intake. Adjusting for sex, age, smoking, extent of CAD at baseline, previous AMI and previous CABG slightly attenuated the risk estimate to 22% (CI 4 - 42). Adding BMI and diabetes mellitus to the model further attenuated the excess AMI risk to 17% (CI -1 - 38) (Table 7).

To test the independent effect of the various choline species, each choline specie was individually added to model 2. Compared to model 2 without the choline species, phosphocholine did not materially change the risk estimate. Free choline and glycerophosphocholine tended to increase it, whereas it was attenuated after adding phosphatidylcholine and sphingomyelin (data not shown).

The same models were thereafter constructed when analyzing the effect of 10 mg increase in each choline specie intake on incidence of AMI, now excluding total choline from the models. Free choline, phosphocholine and glycerophosphocholine had no statistically significant effect on incidence of AMI in any of the models. Intake of phosphatidylcholine and sphingomyelin was positively associated with risk of AMI, although it was attenuated in the multivariate models.

In figure 4 the linearity between total choline intake and AMI was analyzed after adjustment as for model 3. Increasing intake of total choline up to 300 mg/d seemed to be associated with a higher risk of AMI with no excess risk at higher levels of choline intake. However, a clearer linear relationship was observed primarily for phosphatidylcholine and, to

a lesser degree, for sphingomyelin. Intakes of free choline, glycerophosphocholine and phosphocholine do not seem to be associated with risk of AMI.

Table 7. Hazard ratios for incident acute myocardial infarction according to total choline and choline species intake

	Hazard ratio (95% confidence interval)					
	Model 1 ^a	<i>P</i>	Model 2 ^b	<i>P</i>	Model 3 ^c	<i>P</i>
Total choline intake ^d	1.28 (1.09-1.49)	0.002	1.22 (1.04-1.42)	0.013	1.17 (0.99-1.38)	0.051
Free choline ^e	1.04 (0.97-1.11)	0.22	1.03 (0.96-1.10)	0.39	1.02 (0.95-1.09)	0.57
Phosphatidylcholine ^e	1.05 (1.02-1.08)	0.001	1.05 (1.02-1.08)	0.001	1.04 (1.01-1.07)	0.005
Phosphocholine ^e	1.15 (0.93-1.42)	0.19	1.11 (0.89-1.39)	0.33	1.09 (0.88-1.35)	0.44
Sphingomyelin ^e	1.41 (1.11-1.80)	0.004	1.33 (1.05-1.68)	0.02	1.27 (0.99-1.62)	0.053
Glycerophosphocholine ^e	1.03 (0.99-1.07)	0.15	1.01 (0.97-1.05)	0.64	1.01 (0.96-1.05)	0.74

HR, hazard ratio; CI, confidence interval.

^a Adjusted for energy intake.

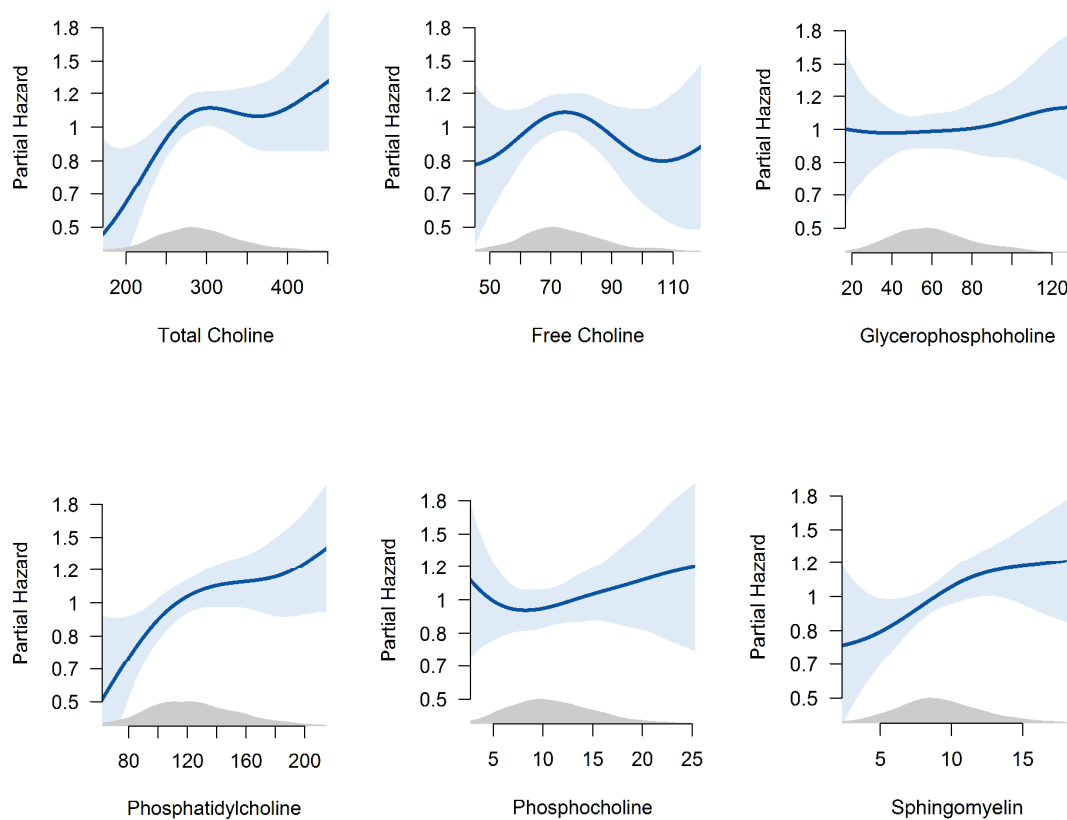
^b Adjusted for model 1 plus age, sex, previous AMI, previous CABG, smoking and extent of CAD at baseline.

^c Adjusted for model 2 plus body mass index and diabetes.

^d per 100 mg/day increase

^e per 10 mg/day increase

Figure 4. Association between intake of choline and acute myocardial infarction



The model GAM was adjusted for energy intake, age, sex, previous AMI, previous CABG, smoking and extent of CAD at baseline, BMI and diabetes. The x axis represents choline intake in a population of 2019 patients with SAP.

5. Discussion

In this large cohort of patients with SAP, having a higher choline intake was associated with increased risk of AMI. The intake of different choline-containing species had varying effects on AMI risk. Free choline, phosphocholine and glycerophosphocholine intake was not associated with AMI. But intake of phosphatidylcholine and sphingomyelin was positively associated with risk of AMI.

Total intake of choline was independent of age, gender, prior AMI, coronary vascularization or extent of CAD at baseline. A high intake was however with several established CVD risk factors including, hypertension and diabetes, whereas no association was observed with lipid levels or plasma choline. A high intake was associated with lower tHcy and higher TMAO concentrations. Higher intake of choline was positively associated with intake of fiber, protein, vegetables, fruits and berries, and alcohol, and negatively associated with carbohydrate and SFA.

5.1 Methodological Discussion

5.1.1 Dietary Assessment

In this study, the aim was to measure dietary choline in patients with SAP. For that purpose, it was applied an FFQ created for epidemiological studies of diet and health, whose goal was to measure average food intake of individual Norwegian adults (Nes et al., 1992). Indeed, the purpose of FFQs is generally to estimate intake of food over longer periods (Willett, 2013). In case of diseases that require a significant amount of time to progress, measuring habitual intake of food is more relevant than food intake for one or few days. Besides that, it can be very useful when measuring population-wise dietetic ingestion for using as a basis for nutritional policy (Andersen et al., 1999). It is also possible to reduce costs when they are self-administered (Willett, 2013).

Another important goal with an FFQ is to rank subjects in their absolute intake. Food items must be chosen firstly, based on their consumption by a considerable number of individuals, secondly, they must have a significant amount of the nutrient of interest, and thirdly, its consumption must vary from person to person to allow ranking among the study participants. A sum of the three aspects named above will contribute for the variance between persons in the nutrient intake (Willett, 2013). Importantly, the present FFQ contains the foods that usually have been reported to be the main sources of choline in the diet as bread, meat,

fish, egg, milk and dairy. Still, changes in this FFQ, as including other choline-rich food items (as cottage cheese, cream cheese, pork products and soya beans), having exclusive questions for food items as spinach and shrimp, locating peanuts in another food group than “dessert, cakes and candies” are examples of alterations that could have yielded other results regarding total choline intake in this population. Nevertheless, the estimates of daily intakes among our patients are aligned with the estimates obtained in other studies in western population.

Another important aspect of a FFQ is that the chosen food items it contains must match the cultural background of the population of study (Willett, 2013), which was the case of the applied FFQ. Also, when assessing nutrient intake, it is decisive to consider characteristics of the food such as physical state (powder, purée, liquid for instance), preparation method (baking, frying, boiling) or vitamin fortification since these aspects are important determinants of nutrient content in the investigated food items, and not considering them is unfit for the purpose of nutrient intake assessment (Vennemann et al., 2015). Regarding choline content in food items some points must be considered. Firstly, total choline content may remain the same when comparing raw or treated food, but the choline species may change. Free choline or phosphatidylcholine content may increase, for instance, while others decrease in proportion, depending on the preparation method (Zeisel et al., 2003). Secondly, choline may have different bioavailability from foods (Emmert and Baker, 1997). Thirdly, hydrosoluble and liposoluble choline species have different absorption mechanisms and metabolism (Zeisel et al., 2003). These aspects may be important for the final effect choline intake has on health as the different choline species are differently associated with AMI as showed in the present study.

Moreover, the fact that our subjects were patients with a clinical diagnose, and occasionally knew the severity of their respective diseases, may have influenced their eating habits prior to the study. Overestimation or underestimation of nutrient intake may be a source of bias in FFQs, and it affects nutrient intake estimation (Mirmiran et al., 2006). Underreporting of choline intake at the individual level may have impaired the risk estimates of choline-disease relation, and adding the fact that the FFQ was not built for measuring choline, may have given attenuated results. To help controlling for these factors in the group as a whole, nutrient intake was adjusted for energy intake. Adjusting nutrient intake for energy is important when energy intake and disease are associated as they are in the case of CVDs (Mirmiran et al. 2006). This adjustment of nutrient intake allows for control of the confounding effect total energy intake may have on disease risk, and it also controls for

extraneous variations in nutrient intake due to very high and/or very low energy intake (Willett et al, 1997)

To estimate choline in food items, it was primarily used the American food database issued by USDA. It cannot be dismissed that this may have accounted for some estimate differences, and that it may have represented a source of estimation error. This source of error can affect the estimated mean intake of the dietary variable at the individual and population level (Slimani et al., 2007). This would, however, have a bigger impact on absolute intake rather than on the ranking of individuals from low to high intake which is more important for the current study. Choline supplements were not included in the FFQ. There was little knowledge about choline in supplements consumed by the population then.

In other words, the role that dietary choline may play in AMI risk depends on the capability of the study to account for all probable confounding by associated dietetic and non-dietetic risk factors (Kritchevsky and Kritchevsky, 2000).

5.1.2 Study Population and Design

Participants of the present study comprehend a homogeneous cohort of patients with angiographically established CAD originally taken from a larger population recruited for the WENBIT study. The current study uses dietary information collected prior to any possible AMI event, and participants are followed-up for the end-points, so this is a prospective cohort. Although prospective cohort studies may be more expensive than other designs and also be time-consuming, this design is considered an efficient tool to study the relation between exposure (dietary choline) and outcome (AMI) (Setia, 2016). In a prospective cohort study it is possible to study a potential causative relation between exposure variable and outcome as their chronologic order is known and the study population is free of the outcome at baseline (Mann, 2003). For the present study, there were collected an extensive amount of variables, which allows for control of many confounding variables.

After the exclusion criteria (extreme caloric intake, and ACS), the study population was composed by a homogeneous group of individuals with diagnose of CVD. For this reason, our results limit to this type of group and cannot be generalized to the rest of the population.

5.1.3 Epidemiological Statistics

Total caloric intake has been inversely associated with CVD (Willett et al., 1997). In this case, nearly all nutrients will be associated with disease risk in the same manner (Willett et al., 1997). This is because lower energy intake is associated with lower food intake, which, by its turn, leads to lower nutrient intake. Overreporting food intake and factors that influence energy intake, and consequently nutrient intake, as physical activity for instance (Willett et al., 1997) may work as a confounder (Rhee et al., 2014). For control of the confounding effect of energy intake, nutrient residual model was applied before all statistical analyses took place. Regression analyses generated residuals of nutrient intake, which were used to estimate intake of dietary choline independent of energy intake. This fact may be due to chance, but dietary patterns and demographic variation in a population may also generate different degrees of correlation between nutrient intake and energy intake (Rhee et al., 2014), which reinforces the importance of adjusting for total energy intake.

Total energy-adjusted choline intake was categorized in quartiles. This allowed estimation of association between baseline categories for different exposure categories, which is positive when, in a real situation, higher or lower intakes affect health negatively (Willett et al., 1997).

Some variables present missing values. There were though a low number of missing values, and they are not likely to have substantially influenced results.

5.2 Discussion of Results

5.2.1 Choline Intake and Baseline Dietary Intake

The presented FFQ issued choline intake estimates similar to what other studies have observed (Detopoulou et al., 2008; Dalmejer et al., 2008; Bidulescu et al., 2007; Cho et al., 2006). While some other studies have estimated higher total choline intake for men (Millard et al., 2016) or for both sexes (Nagata et al., 2015; Fischer et al., 2007; Fischer et al., 2005). These found differences can in part be explained by high consumption of seafood in one study (Nagata et al., 2015), and of eggs among African-Americans men (Millard et al., 2016), which could probably be explained by another eating pattern in their North-American cohort. And in the other two studies (Fischer et al. 2007 and Fischer et al., 2005), the difference could be explained by the fact that subjects were housed at the local University Clinical Research Center and their food intake was observed during the study period. This may have

influenced their *ad libitum* food intake (Robinson et al, 2015). Improved measurement tools and techniques may have been applied in their estimation of choline intake. In addition, choline intake may vary according to other determinants such as ethnicity and country (Slimani et al., 2002).

In the current study population, mean energy-adjusted total choline intake was within what the EFSA estimated to be the average intake of choline (269 to 468 mg/day) among adults after analyses of 12 surveys in 9 countries of the European Union (EFSA, 2016). However, the mean energy-adjusted choline intake in the highest quartile in the present study was below the estimated AI recommended by the EFSA (440 mg/d for adults). Choline intake was neither associated with age or gender in the present study, as observed in another population (Millard et al., 2016).

So far, overall dietary intake of choline in most studied populations has been lower than current established AIs, with some exceptions (Nagata et al, 2015; Fischer et al., 2007; Fischer et al., 2005). Still, the estimated “low” average intake among free-living individuals seems to satisfy the human body’s daily requirements for choline (Cho et al, 2006), in spite of conceivable presence of SNPs that might alter (increase or decrease) choline requirements.

Further, among energy-giving nutrients, higher intake of choline was negatively associated with intake of SFA and total carbohydrates, and positively associated with higher intake of total fiber, protein, vegetables, fruits and berries, and alcohol. This could suggest that choline intake in this population was associated with a healthier eating pattern, and food choices are of substantial importance for the quantity of choline one eats. However, adjustment for dietary intake did not materially affect the results.

5.2.2 Choline Intake and Acute Myocardial Infarction

A higher intake of energy-adjusted total choline was associated with higher risk of AMI in patients with SAP. Increased intake of energy-adjusted phosphatidylcholine was associated with risk of AMI, and increased intake of energy-adjusted sphingomyelin was associated with increased risk of AMI in the crude model, and was borderline statistically significant in the fully adjusted. Energy-adjusted free choline, phosphocholine and glycerophosphocholine had no statistically significant effect on risk of AMI in any of the models. Similarly, Zheng and colleagues also found a higher intake of phosphatidylcholine intake to be associated with increased risk of all-cause mortality, especially CVD-specific mortality in healthy individuals (Zheng et al., 2016). Those associations were stronger in diabetic patients (Zheng et al,

2016). Their study had a large study population, and a long follow-up with biennially dietary assessment. Remarkably, the richest sources of both phosphatidylcholine and sphingomyelin are animal products, according to the table content issued by USDA (Patterson et al., 2008). Perhaps intakes of both choline species are correlated via dietary sources, and this could explain the association between sphingomyelin and AMI found in the present study.

Millard and colleagues, (2016) analyzed association between dietary choline and betaine using a FFQ, and incident CHD, ischemic stroke and CVD in African-Americans. They found a decreased risk of incident ischemic stroke with higher choline intake (Millard et al., 2016).

Other studies did not find the same association (Nagata et al., 2015; Dalmeijer et al., 2008; Bidulescu et al., 2007). In a healthy Japanese population, higher intakes of choline and betaine were not associated with cardiovascular mortality risk (Nagata et al., 2015). Seafood was a good source of choline and betaine, among PUFAs and other nutrients that may exert protective effect in the cardiovascular health. Although the authors adjusted their analyses for seafoods, they remarked that they could not disregard the protective effect of PUFA or other nutrients in seafoods on CVD mortality risk. In addition, they found that higher sphingomyelin intake increased the risk of mortality from CHD in men, and an inversed association between phosphocholine intake and risk of mortality from hemorrhagic stroke in women (Nagata et al., 2015).

Dalmeijer et al., 2008, found no association between regular intake of choline among Dutch postmenopausal women and CVD risks after adjusting for confounders. The authors suggest that the narrow intervals of choline intake used could explain their results (Dalmeijer et al, 2008), but they are not considerably different from the intervals of choline intake used in the present study.

Bidulescu et al. assessed intakes of choline or betaine at baseline with a 66-item FFQ in a large biracial cohort of men and women with no previous CHD. After controlling for multiple risk factors, individuals at highest intake levels had 22% higher risk of experiencing CHD, and 14% higher risk considering the highest choline and betaine intakes together but those estimates were not statistically significant.

Rajaie and Esmailzadeh, 2011, conclude in their review of 7 cross-sectional and prospective studies on choline and betaine and CVD risk on healthy subjects, that the intake of these nutrients is not associated with CVD incidence, but the long-term consumption has been shown to prevent CVD mortality by decreasing inflammation and other risk factors.

Lastly, total energy-adjusted choline intake was not related to baseline plasma lipid profile, as observed in another study (Dalmeijer et al., 2008). But any possible association between total choline and blood lipids could have been masked by the broad use of statins among our subjects (88.3%). Nevertheless, LDL-C and ApoB-100/ApA1 were significantly higher and HDL-C significantly lower in individuals with AMI compared to those without AMI. There were no differences in TC and in TG. The ApoB/ApA1 ratio is a strong predictor of fatal AMI irrespective of TC and TG as ApoB is present in all atherogenic lipid particles (McQueen et al., 2008; Walldius et al., 2001). But adding blood lipids to our regression model 2 did not materially affect the association between choline intake and the risk of AMI.

Some studies used in this discussion included only subjects with established CVD whereas others included only those who, at baseline, did not have CVD. Participants in the current study had comorbidities that were important risk factors, but our analyses were adjusted for them.

5.2.3 Plasma levels of Metabolites and AMI

The mean plasma of choline measured in the present study is in harmony with the estimates given by IOM, 1998, that is, 7 to 20 $\mu\text{mol/L}$ in adults, most having a concentration of 10 $\mu\text{mol/L}$. It was not observed any association between choline intake and concentration of choline, betaine, or DMG in the plasma. Lack of association might be explained by the rapid absorption of choline and its distribution to the different body tissues, as well as interactions with other dietary nutrients, intestinal conditions, and health and physiologic status, like pregnancy and lactation (Cho et al., 2016).

Plasma choline was higher in patients who had an AMI episode but, the difference was only borderline statistically significant. In another study, plasma choline level, which was similar to the average plasma choline in the present study, was associated with an unfavorable CVD risk profile and plasma betaine was associated with a favorable CVD risk profile (Konstantinova et al., 2008). Choline and betaine plasma could be a reflection of last dietary intake, and changes in diet at a later point of time may attenuate associations between plasma choline and AMI risk.

In this study, mean tHcy was within the normal range of tHcy in the blood (5 - 15 $\mu\text{mol/L}$) (Robinson, 2000). Higher intake of choline was associated with lower tHcy, as observed in other studies (Dalmeijer et al., 2008; Cho et al., 2006). Patients who developed AMI under the study period had significantly higher tHcy than those who did not. But,

adding intervention allocation to model 2 in the analyses risk, did not change risk of AMI. Elevations in tHcy seem to be related to only 10% of CAD risk in the population (Weiss et al., 2002). Moreover, a meta-analysis showed no effect on risk of AMI among individuals with established CVD or renal disease who took B-vitamins and had their Hcy levels lowered (Clarke et al., 2010).

5.2.4 Possible Mechanisms

Choline has been related to cardiovascular (Tang et al, 2013; Wang et al., 2011) and more specifically, atherosclerotic diseases (Wang et al., 2011; Muller et al., 2010) through several mechanisms.

Hcy serves solely as an intermediate in the metabolism of MET, and it must be remethylated (remethylation pathway) or it must be catabolized (transsulphuration pathway) (Blom and Smulders, 2011). Choline deficiency, through scarce betaine, could lead to accumulation of Hcy (Dalmeijer et al., 2008; Bidulescu et al., 2007). While supplementation with choline and betaine (Lee et al., 2010) or phosphatidylcholine (Olthof et al., 2005a) is capable of lowering Hcy concentrations. It is well established that B vitamins have the capacity to reduce levels of Hcy (Weiss et al., 2002) by promoting remethylation or cleavage of Hcy (Bertoia et al., 2015). Increased plasma Hcy would disturb the vascular endothelium, elevating atherogenesis (Weiss et al., 2002). Elevated levels of Hcy in the plasma are associated with higher risks of developing vascular disease (Robinson, 2000) independent of other risk factors (Clarke et al., 1991).

Higher intake of choline has been associated with decreased circulating levels of inflammatory markers, such as CRP, interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF- α) (Detopoulou et al., 2008). Rajaie and Esmailzadeh, 2011, conclude in their review of 7 cross-sectional and prospective studies on choline and betaine and CVD risk on healthy subjects, that the intake of these nutrients is not associated with CVD incidence, but the long-term consumption has been shown to prevent CVD mortality by decreasing inflammation and other risk factors. However, it was not found association between total energy-adjusted choline intake and CRP, and adding CRP to hazard risk analyses did not affect the effect of choline intake on risk of AMI.

Choline is also hypothesized to affect CVD via the action of TMAO. TMAO augments macrophage cholesterol content and foam cell formation in mouse models (Wang et al., 2011). Foam cells play a major role in the progress of atherosclerosis (Valledor et al.,

2015). TMAO also diminishes reverse cholesterol transportation (Koeth et al., 2013), and alters the metabolism of bile acid and sterol transporters in the liver and intestine (Velasquez et al., 2016). Wang and colleagues observed a dose-response relationship between TMAO and angiographic measures of atherosclerotic plaque in humans (Wang et al., 2011). High plasma choline (Wang et al., 2014; Koeth et al., 2013; Wang et al., 2011), high plasma betaine (Wang et al., 2014; Wang et al., 2011), high plasma carnitine (Koeth et al., 2013) have been found to be associated with higher CVD risk only when TMAO concentration was high. In the present population, plasma concentration of TMAO was higher across quartiles of choline intake, but there was no significant difference in TMAO serum levels between patients with AMI and patients who did not have AMI. Importantly, TMAO is excreted via urine effectively (Tang et al., 2013; Wang et al., 2011). In such way, TMAO clearance may protect against CVD (Tang et al., 2013). Velasquez and colleagues in their review article suggest that TMAO could instead be a marker of stress rather than a mediator of disease though (Velasquez et al., 2016). The literature about TMAO and CVDs remains controversial still (Velasquez et al., 2016).

Elevated intakes of choline and betaine have also been associated with altered blood lipids (Olthof et al., 2005b), what may elevate risk of CVD. Olthof and colleagues administered choline and betaine to healthy subjects, and showed that choline supplementation may increase TG, and that betaine may increase LDL-C and, consequently, TC (Olthof et al., 2005b). The participants received though 6g of betaine and 2.6 g of choline per day, that is far above the estimated average choline intake in epidemiological studies so far. They propose that the export of lipids from the liver via VLDL-C increase, as its precursors (choline and betaine) are found abundant intracellularly due to elevated intake (Olthof et al., 2005b). Although there were observed some differences in blood lipids between those who had AMI and those who did not, especially Apob/Apa1 ratio, blood lipids did not modify the estimated risk association between dietary choline and AMI when added to model 2.

6. Conclusion

In our study, we found that there was an increased risk of experiencing AMI for each 100 mg increase in choline intake among Norwegian patients with SAP.

The exact mechanism through which higher choline intake is associated with AMI events in the present population is uncertain, and it may be multifaceted due to the ubiquitous use of choline in the human body. Nevertheless, due to the prospective nature of the present study, we could observe a potential causal effect between high choline intake and incidence of AMI. Although no conclusion can be made, the present results add valuable knowledge to future research.

7. Future Perspectives

Creating a Norwegian and a European database for choline content in foods is necessary for more accurate assessment of choline content in foods purchased and consumed in Europe. In addition, divergencies in choline requirement may obscure any possible consistency among studies, and it must be considered in the future. A broader knowledge about how estrogen (and possibly hormonal replacement therapy) and SNPs affect choline necessities will add precious understanding to the varied impact this nutrient have on health.

Additionally, choline metabolism is interrelated with other important pathways that play an important positive or negative role on the development of CVD. In this way, controlling for those variables are of great importance when exploring the effect of choline intake in different populations.

Controlled trials are warranted in order to better comprehend the effects of choline intake in the development of CVDs. Control and intervention groups with choline intakes within or above the current AIs will cooperate defining what higher intakes are in different groups. Moreover, exploring the different effects of free choline and choline species on health and disease is valuable, especially because most of animal foods rich in this nutrient are recommended to be consumed in modest amounts.

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