Association between Dietary Betaine Intake and Circulating Levels of Parameters Related to Choline Oxidation and Lipid Metabolism in Patients with Stable Angina Pectoris

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Abstract

Introduction: Cardiovascular disease (CVD) is among the leading causes of death worldwide, and is strongly influenced by lifestyle. Thus, promoting a healthy lifestyle, which includes having a healthy diet, is among the primary preventive strategies for reducing future risk of cardiovascular events. Elevated circulating total homocysteine (tHcy) is an independent risk factor for CVD. However, Hcy lowering treatment has not proven to reduce mortality or cardiovascular events among patients with CVD, and the mechanism linking tHcy to disease risk has not yet been discovered. Thus, the metabolic pathways surrounding Hcy, including the one-carbon metabolism and the choline oxidation pathway, are very interesting targets for investigation.

Betaine is a micronutrient that is achieved mainly from the diet or endogenously by irreversible mitochondrial oxidation of choline. Betaine lowers tHcy concentrations in the liver and kidney by serving as a methyl donor in a reaction catalyzed by betaine-homocysteine methyltransferase. This reaction represents the first step along a transmethylation pathway, which provides three available methyl groups in the one-carbon metabolism. Betaine is thus considered to be an important modulator of the one-carbon metabolism. Furthermore, betaine-dependent remethylation of Hcy produces methionine. Betaine has thus been hypothesized to affect the hepatic lipid metabolism by producing sufficient amounts of methionine which enters a pathway that ultimately leads to increased synthesis and secretion of lipid-rich very-low density lipoproteins (VLDLs).

Objective: The main aim of the current study was to explore the associations of reported dietary betaine intake with circulating levels of metabolites along to the choline oxidation pathway and lipid related parameters in patients diagnosed with stable angina pectoris (SAP). A secondary aim was to explore potential effect modification by folate status and statin treatment.

Methods: This cross-sectional study was based on the Western Norway B-vitamin Intervention Trial, and included 2026 SAP patients with angiographically verified coronary artery disease, and available dietary data. Average daily dietary intakes during the recent year were assessed by a 169-item food frequency questionnaire. Standard blood laboratory parameters were measured according to routine protocols at the two university hospitals in Western Norway. The associations between reported dietary betaine intake and one-carbon

metabolites, including choline oxidation, were assessed by Spearman´s rank correlations, adjusted for age, gender, total energy intake and statin treatment. Multiple linear regression was used to explore the relationship between reported dietary betaine intake by quartiles and according to the upper decile, and levels of lipid related parameters, and were adjusted for age, gender, body mass index (BMI), total energy intake, current smoking, statin treatment and diabetes. We also evaluated the effect modification by folate status and statin treatment in these linear regression analyses.

Results: Median dietary betaine intake in this cohort was 134 mg/d. The participants (80.4% males) had a median age of 62 years. Unadjusted Spearman correlation analyses revealed a positive association of dietary betaine intake with plasma betaine ($r=0.17$, $p<0.001$), DMG ($r=0.06$; $p=0.006$) and sarcosine ($r=0.12$, $p<0.001$) and an inverse association with tHcy ($r=-0.09$, $p<0.001$). The association with plasma betaine was attenuated by adjusting for age, gender, total energy intake and statin treatment, but none of the other associations were appreciably affected by such adjustment. Furthermore, in the unadjusted linear regression analyses increasing intakes were associated with lower levels of serum LDL-, HDL-, and total cholesterol, Apo B and Apo A1. Adjusted multiple linear regression analysis demonstrated no significant associations between quartiles of betaine intake and lipid related parameters and no effect modification with folate status or statin treatment was observed. However a particular high intake corresponding to the upper decile was associated with significantly higher plasma HDL-cholesterol and lower Apo B levels. Subgroup analyses revealed this association to be restricted to those with a serum folate level above median. Furthermore, participants with particular high betaine intake and who were treated with statins had significantly lower plasma LDL-cholesterol and Apo B levels, while no such associations were seen in those not using statins.

Conclusion: In the present study among 2026 patients with SAP, there was a significant positive association between reported dietary betaine intake and circulating betaine, DMG and sarcosine, and a significant inverse association with circulating tHcy. A particular high betaine intake corresponding to the upper decile was associated with significantly higher HDL-cholesterol and lower Apo B. A beneficial effect of high betaine on the lipid profile tended to be restricted to patients with high serum folate and to those treated with statins.

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Abbreviations

ACS	Acute coronary syndrome
Apo A1	Apolipoprotein A1
Apo B	Apolipoprotein B
BADH	Betaine aldehyde dehydrogenase
BGT1	Betaine/γ-aminobutyric acid transporter
BHMT	Betaine-homocysteine methyltransferase
BMI	Body mass index
CAD	Coronary artery disease
CHD	Coronary heart disease
CHDH	Choline dehydrogenase
CVD	Cardiovascular disease
DM	Diabetes mellitus
DMG	Dimethylglycine
eGFR	Estimated glomerular filtration rate
EI	Energy intake
FFQ	Food frequency questionnaire
GC-MS/MS	Gas chromatography coupled to tandem mass spectrometry
Hcy	Homocysteine
HDL	High-density lipoprotein
LC-MS/MS	Liquid chromatography coupled to tandem mass spectrometry
LDL	Low-density lipoprotein
LVEF	Left ventricular ejection fraction
MI	Myocardial infarction
mRNA	messenger RNA
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
RNA	Ribonucleic acid
SAH	S-adenosyl homocysteine
SAM	S-adenosylmethionine
SAP	Stable angina pectoris

TG	Triacylglycerol
tHcy	Total homocysteine
THF	Tetrahydrofolate
VLDL	Very low-density lipoprotein
WENBIT	Western Norway B-vitamin Intervention Trial
%TE	Percent of total energy intake
5-mTHF	5-methyltetrahydrofolate

1.0 Introduction

1.1 Cardiovascular disease

Cardiovascular disease (CVD) is a collective term for several pathological conditions in the heart, vascular system or pericardium [1]. CVD remains a leading cause of death in Western countries, causing nearly one-third of all deaths worldwide [2]. The majority of CVDs are pathologies in the arterial circulation, collectively referred to as atherothrombotic CVD, due to their clinical manifestations, often in the form of thromboembolic complications [3]. Atherothrombotic CVD (hereafter referred to as CVD) is usually caused by arteriosclerosis, and includes coronary heart disease (CHD), cerebrovascular disease, diseases of the aorta and peripheral arterial disease [1]. Among the CVDs, CHD is the major cause of CVD related deaths [2].

1.2 Coronary heart disease

CHD occurs when the coronary vessels have a decreased capacity to deliver oxygen-rich blood to the heart [1, 4]. The metabolic changes that occur in tissues due to reduced oxygen delivery are called ischemia, and CHD is therefore often called ischemic heart disease [4]. Ischemia in the heart may typically cause chest pain, known as angina pectoris [5, 6]. Patients are further diagnosed with stable angina pectoris (SAP) if the chest pain symptoms remain stable for usually 60 days, in contrast to unstable angina pectoris [6]. In the majority, however, CHD usually first manifest itself as an acute coronary syndrome (ACS), including unstable angina, acute myocardial infarction (MI), severe arrhythmia or sudden cardiac death [1, 4]. Thus, a large proportion of patients with prevalent SAP includes patients previously suffered and treated from ACS [6]. The prevalence of SAP was estimated to be 17.2 million in Europe and 54.0 million worldwide in 2004 [7].

1.3 Arteriosclerosis

The disease processes of the arterial wall, which includes chronic inflammation and hardening of the arteries is called arteriosclerosis [8]. A main underlying pathophysiologic mechanism of arteriosclerosis development is often so-called atherosclerosis [9], characterized by the presence of an atheroma within the arterial lining. The atheroma includes inflammatory cells

like monocytes and macrophages, which have been turned into so-called foam cells due to a large uptake of cholesterol and other lipids [8, 10]. Recently, such foam cells have been shown also to be of smooth muscle cell origin. Excess foam cell formation or other stimuli of apoptosis may cause the foam cells to rupture, and free cholesterol and cholesterol crystals stimulate the inflammatory process, which may promote so-called plaque rupture, a typical underlying feature of an ACS [4]. Thus, lipid metabolism is crucially involved in arteriosclerosis related clinical events. Elevated plasma cholesterol levels, the oxidation of low-density lipoprotein (LDL) particles and their retention in the arterial intima are considered primary elements of this process [11]. The development is usually slow, but has a high potential of rapid progress.

1.4 Risk factors for CVD

Based primarily on data from a number of large-scale epidemiological observational studies during the last 50 years, factors related to a so-called unhealthy lifestyle like smoking, physical inactivity, unhealthy dietary habits, obesity, diabetes, hypertension and hyperlipidemia are associated with increased risk of CVD [12-19]. In addition, non-modifiable risk factors for CVD are age, gender, and family history, where future risk increases if a first-degree relative have suffered from CVD before the age of 55 years for males and 65 years for females [2]. Due to the strong influence of lifestyle, CVD is included among the typical lifestyle diseases [20]. Thus, promoting a healthy lifestyle, which includes having a healthy diet, is essential in the prevention of this disease, also among those receiving medical treatment for this condition, as recommended by the American Heart Association and the European Society of Cardiology [21, 22].

1.5 Diet and CVD

The association between dietary intake of both saturated fat and trans fat and risk of CHD has been well established [23, 24]. Thus, as part of a healthy diet the American Heart Association recommends dietary intake of saturated fat and trans fat restricted to <7% and <1% of the total daily energy intake (EI), respectively [21]. The same guideline advocates the replacement of saturated fat with mono- and polyunsaturated fat [21, 22], which has previously been shown to significantly reduce risk of CHD [25-27]. However, recent findings from meta-analyses of observational studies and randomized controlled-trials now questions

the established association between intake of saturated fat intake and risk of CHD [23, 28-30]. Moreover, compared to dietary fat, epidemiologic studies on the association between dietary protein intake and risk of CHD are limited, whereas data on overall protein intake from observational and prospective cohort studies are not supportive for such an association [31-33]. Intake of read meat may be associated with elevated risk [32, 34, 35], while intake of soy may be protective [36-38]. Furthermore, dietary intake of carbohydrates with a high glycemic index has been significantly associated with increased risk of CHD, when compared with carbohydrates with a low glycemic index, which do not increase disease risk [39-41].

Dietary intake of various fruits and vegetables, in addition to various types of grains, cereals and dietary fiber is essential in a healthy daily diet, as recommended by the American Heart Association [21], and has been associated with a decreased risk of CVD [42-48]. A recent randomized trial reported that a Mediterranean diet supplemented with extra virgin oil and mixed nuts reduced cardiovascular events [49]. The Mediterranean diet typically includes a high intake of olive oil, fruit, nuts, vegetables, cereals, and fish, while poultry, dairy products and read meat are kept at a moderate level. According to a systemic review, this type of diet appeared to be the most protective diet against CHD [50]. Lastly, lower-than-normal plasma levels of antioxidants as a result of low vitamin intake has been associated with excess risk of CHD [51]. However, according to a recent meta-analysis of randomized controlled trials there is no evidence to support that supplementation with antioxidants or vitamins reduces the increased risk of CVD [52]. This includes B-vitamins, as demonstrated in the two Norwegian studies, the Norwegian Vitamin Intervention Trial and the Western Norway B-vitamin Intervention Trial (WENBIT), whose main aim was to test whether lowering of plasma total homocysteine (tHcy) could slow the progression of CHD and reduce mortality in patients with established CHD [53, 54].

1.6 One-carbon metabolism

An elevated plasma or serum tHcy level, known as hyperhomocysteinemia is regarded as an independent risk factors for CVD [2, 55-60], and has been associated with premature vascular disease [61-65], and with overall risk of mortality among patients with angiographically verified coronary artery disease (CAD) [56, 59]. Hyperhomocysteinemia was assumed to play a causative role in the development of atherosclerosis [55], and may be due to a defect in remethylation or transsulfuration pathways of Hcy metabolism or by increased production

[66, 67]. Because treatment with folic acid lowers tHcy levels and folic acid is a precursor for 5-methyltetrahydrofolate (5-mTHF), which serves as a methyl donor in the remethylation of Hcy, it was hypothesized that treatment with folic acid would reduce CVD incidence and mortality through increased remethylation of Hcy by increasing methionine synthase activity [68]. However, it has later been demonstrated that increased serum folate concentrations reduce tHcy by being an inhibitor of methyltransferases like glycine N-methyltransferase (GNMT) [69], hereby reducing the Hcy production to spare S-adenosylmethionine (SAM) for other methylation reactions, including methylation of RNA, DNA and proteins, and the formation of phosphatidylcholine (PC) [70]. GNMT inhibition has recently been linked with impaired cholesterol export from the liver or peripheral tissues and with atherosclerosis development [71] which could explain the increased rapid progression of CAD observed after folic acid / B12 treatment in WENBIT [72]. Folate has thus been suggested to influence the activity of betaine homocysteine methyl transferase (BHMT) [73], which uses betaine as methyldonor for the alternative remethylation of Hcy.

BHMT is a cytosolic zinc metalloenzyme, expressed at a high level in the liver and kidney, where it catalyzes the transfer of a methyl group from betaine to Hcy, which is converted to dimethylglycine (DMG) and methionine, respectively, as shown in **Figure 1**. Betaine is therefore essential in order to lower tHcy and maintain methionine concentrations [74]. Furthermore, because Hcy also is remethylated by 5-mTHF in most tissues [68], the metabolism of betaine and folate converges at the point where Hcy resides [75, 76]. Moreover, the betaine-dependent remethylation of Hcy in the BHMT reaction represents the first step along a transmethylation pathway, where three methyl groups are made available. After DMG have been synthesized from betaine, it enters the mitochondrion where it is demethylated to sarcosine, also known as monomethylglycine [77]. Sarcosine is subsequently demethylated to glycine, which is further interconverted to serine, degraded by the glycine cleavage system, or exported out of the mitochondria.

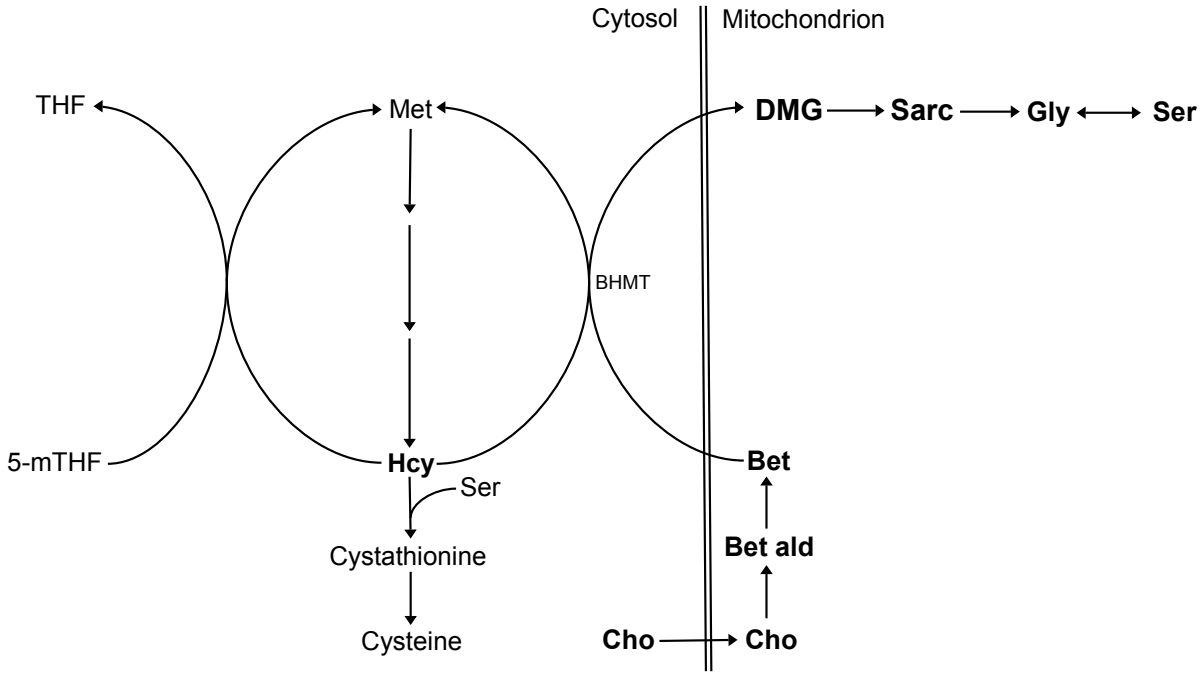

Figure 1: Schematic overview over the one-carbon and homocysteine metabolism. Abbreviations: Cho, Choline; Bet ald, Betaine aldehyde, Bet, Betaine; BHMT, Betaine-homocysteine methyltransferase; DMG, Dimethylglycine; Sarc, Sarcosine; Gly, Glycine; Ser, Serine; Met, Methionine; Hcy, Homocysteine; THF, Tetrahydrofolate; 5-mTHF, 5-mehtyltetrahydrofolate.

1.7 Betaine

1.7.1 Dietary sources

Betaine (N, N, N-trimethylglycine) is a small methyl derivate of the amino acid glycine, containing three chemically reactive methyl groups [75]. It is often referred to as glycine betaine, from now on denoted as betaine, which originally was discovered in sugar beet (Beta vulgaris) [78]. Betaine is abundant in microorganisms, plants and animals and is found in a huge variety of dietary sources [79-82]. Important sources of betaine are plant-based foods, especially cereal foods, such as refined and whole grain wheat, as shown in **Table 1** [82-84]. Because betaine is synthesized *de novo* by the irreversible mitochondrial oxidation of choline, it can also be obtained from choline-containing foods [67]. In 1998, the Food and Nutrition board of the Institute of Medicine recognized choline as an essential micronutrient, and recommended an Adequate Intake of 550 and 425 mg/d for men and women, respectively [85, 86] Even though betaine is an important nutrient, it is not considered as an essential micronutrient, and as far as we know, no official recommendations of a daily intake has been

made [84]. However, dietary intakes have been estimated to range between 100 and 300 mg/d in Europe and the US [87-91].

Table 1: **Betaine content in selected foods**[1]

Food item	Betaine content (mg/100g)
Wheat germ	410
Cereals, all bran	360
Wheat bran	320
Beets, canned	260
Spaghetti, dry	140
Rye flour, dark	150
Spinach, raw	130
Wheat bread	85
Fish, portions and sticks	45
Sweet potato	35
Sunflower seeds kernel, dried	35
Shrimp, canned	23
Nuts, cashew nuts	11
Beef and pork products	0.2 – 17.0

[1] Collected from Patterson, K. et al. 2008, *USDA database for the choline content of common foods. Release two.*

1.7.2 Biochemistry

Betaine is a zwitterionic quaternary ammonium compound at neutral pH, giving it the property of being a highly polar and lipophobic compound, which is easily soluble in water and almost insoluble in various organic solvents [67, 78]. Betaine can be achieved trough the diet from a wide range of animal and plant sources, or by *de novo* synthesis in tissues from choline [84]. Following ingestion, betaine is absorbed through the small intestine via the duodenum by active Na^+ or Cl^- coupled transport, and by passive Na^+ independent transport systems, and is assumed to have a very high bioavailability close to 100% [67, 75]. Because betaine is an N-methylated amino acid, the active transport is carried out by amino acid transport systems, predominantly by betaine/ γ-aminobutyric acid transport and amino acid transport system A [92]. *De novo* synthesis mainly occurs in the liver and kidneys, from choline by mitochondrial oxidation in a two-step enzymatic reaction, as demonstrated in **Figure 2** [93]. First, choline is oxidized to the intermediate substrate betaine aldehyde by the mitochondrial enzyme choline oxidase, also known as choline dehydrogenase (CHDH). The enzyme is a flavoprotein localized in the inner mitochondrial membrane, where it is a part of the respiratory chain [94]. For each molecule of choline oxidized, two molecules of adenosine triphosphate are generated [93]. In the second enzymatic reaction, betaine aldehyde is further oxidized to betaine by a second mitochondrial enzyme, betaine aldehyde dehydrogenase

(BADH), which uses vitamin B3 (nicotinamide adenine dinucleotide) as a cofactor. This two-step enzymatic reaction of choline to betaine is irreversible [95].

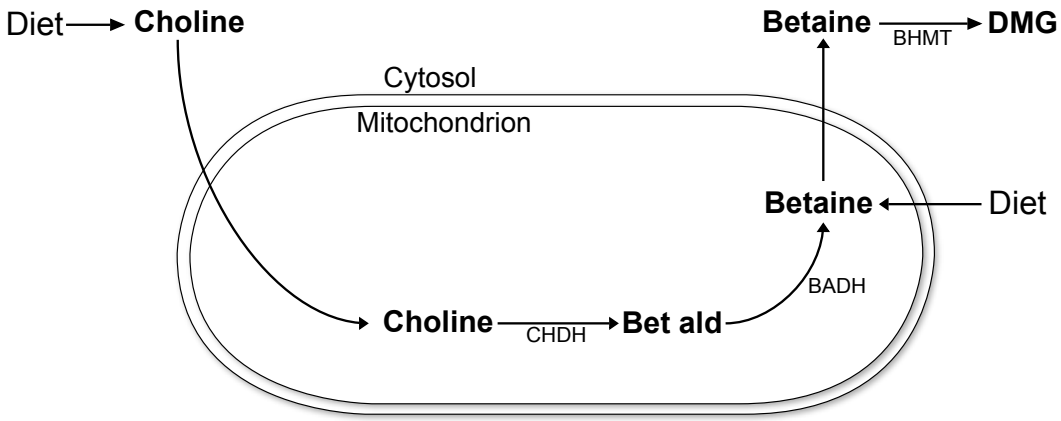

Figure 2: Endogenous betaine synthesis. Abbreviations: CHDH, Choline dehydrogenase; Bet ald, Betaine aldehyde; BADH, Betaine aldehyde dehydrogenase; BHMT, Betaine-homocysteine methyltransferase; DMG, dimethylglycine.

1.7.3 Biological function

In mammals, betaine has two principal physiological roles, which include being an organic osmolyte and a methyl donor. As a methyl donor, betaine participates in the remethylation of Hcy, catalyzed by BHMT in the liver and kidney, which forms methionine and DMG from Hcy and betaine, respectively [73, 75]. In the liver, this pathway is considered to be responsible for about 50% of the Hcy methylation capacity, implying that betaine is of great importance concerning the formation of methionine and maintaining its concentrations in this organ [78]. Furthermore, the activity of BHMT is suggested to be osmoregulated [96]. Thus, the roles of betaine as an osmolyte and as a methyl donor depend upon each other. When intracellular concentrations of osmolytes need to be maintained, the expression of BHMT is downregulated [96, 97], thereby reducing the catabolism of betaine through the transmethylation pathway, making fewer methyl groups available in the one-carbon metabolism [78].

As an organic osmolyte, betaine accumulates in most tissues to regulate cell volume and modulate intracellular osmolarity, and thus protects cells against osmotic stress when

exposed to hypertonic solutions, by replacing inorganic salts [67, 78, 98]. The intracellular concentration of betaine is therefore believed to be much higher than the extracellular concentration [99]. The osmoprotective role of betaine is especially important in the renal medullary cells, which is frequently exposed to solutions with high salinity [100, 101]. The accumulation of betaine occurs via the betaine/γ-aminobutyric acid transporter (BGT1), originally identified in the kidneys, where it is located in the basolateral plasma membrane of the medullary epithelial cells, and has since been reported to be expressed in many tissues [102, 103]. The betaine transporter BGT1 is osmoregulated, and as a response to hypertonic stress, the transcription of the BGT1 gene is activated, followed by increased synthesis and recruitment of the transport protein to the plasma membrane. This allows betaine to rapidly accumulate in the cells and compensate cell shrinkage by the retention of water [103]. When the cells are no longer exposed to hypertonic stress, betaine is rapidly removed by an increase in efflux trough specific pathways, while the influx through BGT1 is reduced [101]. The osmoprotective role of betaine also includes its ability to protect intracellular enzymes and proteins against osmotic stress and higher temperatures, by enhancing protein stability, which are of great importance in the renal medullary cells, where betaine have been reported to counteract the denaturing effect of urea [78].

1.7.4 Betaine and lipid metabolism

Dyslipidemia is disturbance in the lipid metabolism, which is characterized by low serum HDL-cholesterol, elevated plasma TG levels, referred to as hypertriglyceridemia, and elevated plasma LDL-cholesterol levels [104], referred to as hypercholesterolemia [15]. Dyslipidemia are together with insulin resistance and hypertension among the characteristic features of the metabolic syndrome often observed in obese subjects, and also a risk factor for CVD [105].

Betaine supplementation (normally 3-6 g/d) among healthy individuals is believed to affect the lipid metabolism in the liver, which is the major site of lipid metabolism in the body [106, 107]. The hepatic synthesis and secretion of VLDL particles are essential for the transport and delivery of triacylglycerols (TGs) and cholesterol esters from the liver to extra hepatic tissues throughout the body via the circulation [108]. The VLDL particles are especially rich in TGs [109], which are being hydrolyzed by the activity of lipoprotein lipases when arriving at the target cells, leading to fatty acid and glycerol release and the subsequent

conversion of the VLDLs to cholesterol ester-rich LDL particles [15, 107]. The LDL particles then deliver the remaining cholesterol to the peripheral tissues. In contrast high-density lipoproteins (HDLs) are responsible for the transport of cholesterol from extra hepatic tissues back to the liver [108], known as the reverse cholesterol transport.

The hepatic ability to synthesize lipoproteins depends on the function of apolipoproteins, which are structural proteins with lipid-binding properties, where apolipoprotein B (Apo B) and apolipoprotein A1 (Apo A1) are needed in order to synthesize VLDL and HDL particles, respectively [107, 110, 111]. Formation of the mature VLDL particle takes place in the rough endoplasmatic reticulum membrane, and involves the association of Apo B with a TG droplet, which is subsequently encapsulated by a monolayer of phospholipids [109, 110, 112]. The outer monolayer of VLDL particles consist mainly of the phospholipid PC and the apolipoprotein Apo B [110]. In the liver, formation of PC is formed either through the phosphatidylethanolamine (PE) methylation pathway, with SAM as a methyl donor [107], or from free choline [113], and is assumed to play an important role in hepatic VLDL synthesis and secretion [95, 109, 114].

Because betaine is used to form methionine, which is subsequently converted to SAM, betaine supplementation (4-6 g/d) among healthy individuals is suggested to increase the SAM/S-adenosylhomocysteine (SAH) ratio, which further leads to enhanced PC synthesis [106, 107]. In this manner, betaine is hypothesized to affect the lipid metabolism in the liver by increasing the VLDL secretion through an increase in PC synthesis, as shown in **Figure 3**, which further leads to increased TG and LDL-cholesterol levels in plasma, while HDL-cholesterol levels remains unaffected [106]. Furthermore, betaine supplementation together with methionine restriction in rats have been reported to induce the transcription of hepatic BHMT mRNA, compared to methionine restriction alone, followed by an increase in the transcription of Apo B mRNA, which resulted in increased VLDL secretion and a reduction in liver TG concentrations [115].

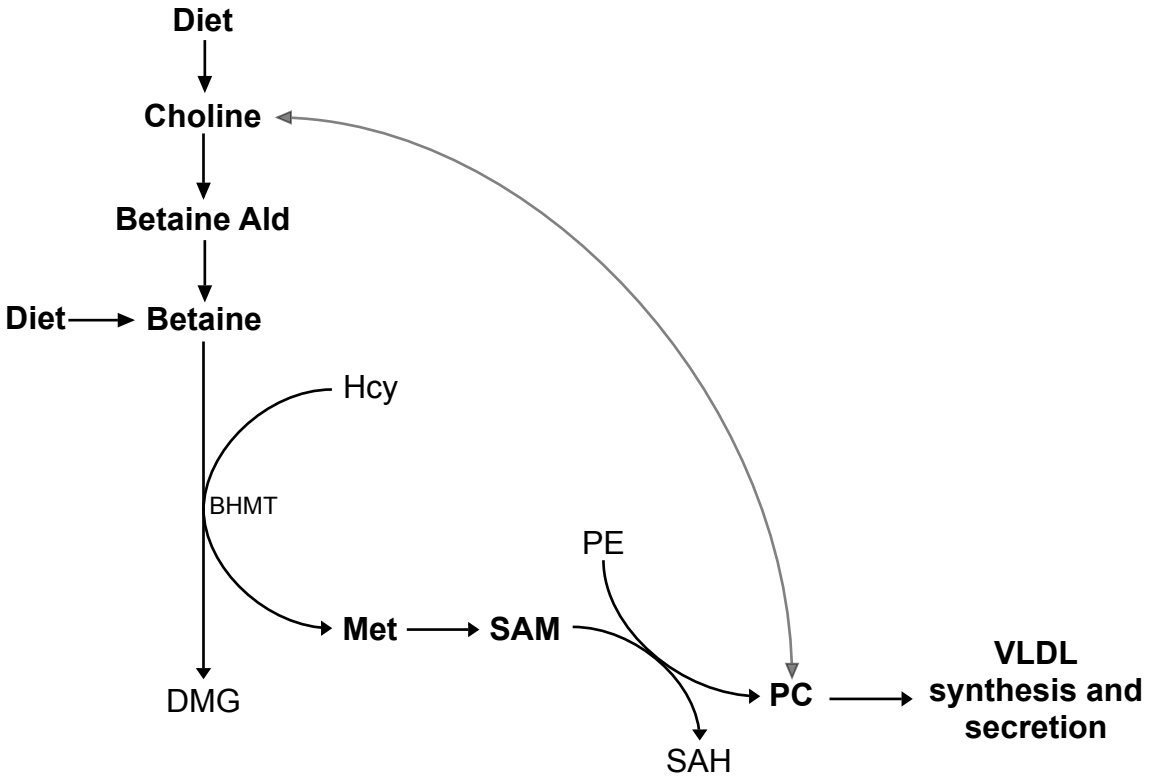

Figure 3: Proposed mechanism of action for the association between betaine and lipid metabolism in the liver. Abbreviations: Betaine Ald, Betaine aldehyde; DMG, dimethylglycine; Hcy, homocysteine; BHMT, betaine-homocysteine methyltransferase; Met, Methionine; SAM, S-Adenosylmethionine; SAH, S-Adenosylhomocysteine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; VLDL, Very-low density lipoprotein.

2.0 Aims

2.1 Overall aim

The overall aim was to investigate the association of reported dietary betaine intake with circulating levels of metabolites along the choline oxidation pathway and serum levels of lipid related parameters in patients with SAP.

2.2 Specific aims

1. To examine the relationship between dietary intake of betaine and circulating levels of choline, betaine, and related one-carbon metabolites.

2. To explore the association between dietary intake of betaine and serum levels of parameters related to lipid metabolism.

3. A secondary aim was to evaluate effect modification by folate status and statin treatment on the association between dietary intake of betaine and lipid metabolism.

3.0 Materials and methods

3.1 Study design and population

The participants included in this study represent a subpopulation of patients from The Western Norway B Vitamin Intervention Trial (WENBIT). WENBIT was a prospective, randomized, double-blind, placebo-controlled secondary prevention study of the clinical effects of B vitamin treatment in patients who underwent coronary angiography for suspected CAD or aortic valve stenosis [54]. Patients diagnosed with cancer, suffering from mental illness, abusing alcohol, participating in other studies, or unavailable for the follow-up appointment were excluded from participating in the trial. The WENBIT study population consisted of 3090 randomized patients, and included both men and women aged >18 years, of which 2121 (68.6%) were randomized at Haukeland University Hospital and 969 (31.4 %) at Stavanger University Hospital between 1999 and 2004. The flow of randomized patients from WENBIT selected for the current study is shown in **Figure 4**.

A food frequency questionnaire (FFQ) was handed out to a majority of WENBIT participants at the day of recruitment, and was returned by 2484 [116]. Patients with more than one blank page (n=19) and those reporting a very low or high total daily energy intake (EI) (n=53) (women reporting <3000 and >15000 kJ/d, and men reporting <3300 and >17500 kJ/d) were excluded. In addition, 10 patients were excluded due to missing blood parameters of choline, betaine and DMG. Furthermore, 337 patients (14.0%) with acute coronary syndrome, and 39 patients (1.6%) with aortic valve stenosis were excluded from the current analysis. Thus, the final study population consisted of 2026 individuals (84.3%) with suspected stable angina pectoris (SAP) diagnosed with CAD at baseline coronary angiography.

3.2 Ethical statement

All patients gave their written consent to participate. The study was in accordance with the principles of the Declaration of Helsinki, and approved by the Regional Committee of Medical Research Ethics, the Norwegian Medicines Agency, and the Data Inspectorate.

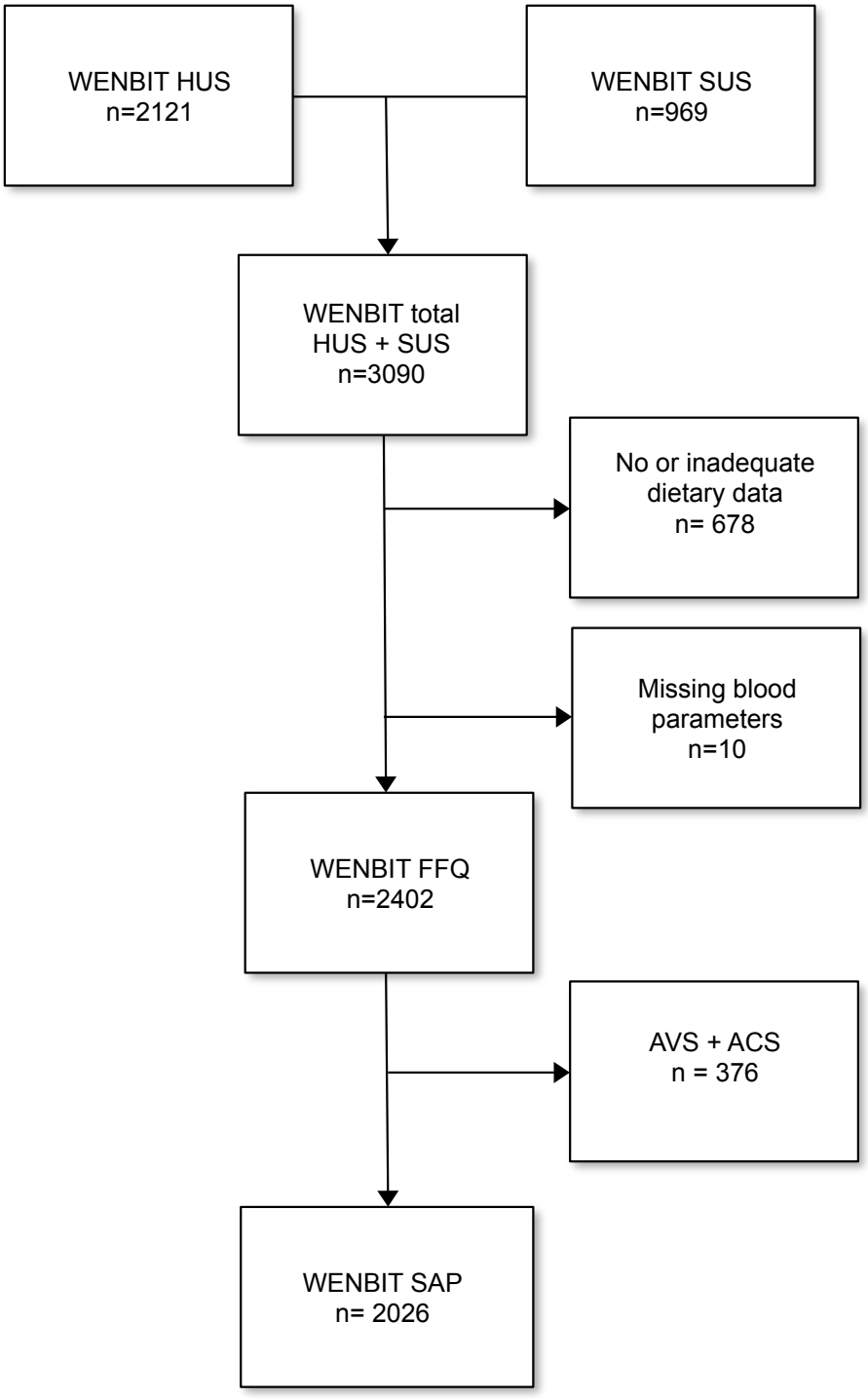

Figure 4: Flow chart of randomized patients from WENBIT to current study population. Abbreviations: WENBIT, Western Norway B Vitamin Intervention Trial; HUS, Haukeland University Hospital; SUS, Stavanger University Hospital; FFQ, Food Frequency Questionnaire; ACS, Acute coronary syndrome; AVS, Aortic valve stenosis; SAP, Stabile Angina Pectoris.

3.3 Baseline data

Information about medical history, lifestyle risk factors, and medications were collected from each participant at baseline and verified against hospital records [54]. Clinical and anthropometric data were collected, measured and assessed by study personnel. Those who received medical treatment for hypertension were defined as being hypertensive. Patients were defined as having diabetes mellitus DM (type 1 and 2) according to preexisting diagnosis, when having a baseline fasting serum glucose ≥7.0 mmol/L or a non-fasting serum glucose ≥11.1 mmol/L, or when having a baseline HbA1c ≥ 6.5%. Left ventricular ejection fraction (LVEF) (%) was determined by ventriculography or echocardiography, where values below 50% were considered to be impaired. The extent of CAD was determined by the use of angiography in order to detect the number of stenotic vessels among the patients, which ranged between 0 and 3, where 0 was defined as no or non-significant CAD, and 1-3 was defined as having a single, double or triple vessel disease, respectively [54]. Estimated glomerular filtration rate (eGFR) was determined by use of the Chronic Kidney Disease Epidemiology Collaboration creatinine equation [117]. Smoking status was determined by self-reported current smoking, including those who quit within the last month, and those with plasma cotinine > 85 nmol/L [118].

3.4 Biochemical analyses

Routine analysis of standard blood parameters was performed directly in fresh blood samples at the two university hospitals in Western Norway, while separate blood tubes were stored in a biobank at -80°C until analysis. Analyses of choline, betaine, the one-carbon metabolites tHcy, DMG, sarcosine, glycine and serine, in addition to cotinine, C-reactive protein and glycated hemoglobin (HbA1c) were performed at Bevital A/S, in Bergen, Norway. All analyses were based on plasma, except sarcosine (serum) and HbA1c (whole blood). The metabolites tHcy, sarcosine, glycine and serine were quantified by gas chromatography, while choline, betaine and DMG, including tHcy, were quantified by liquid chromatography. Both quantificantion methods were coupled to tandem mass spectrometry. Tina-quant reagent kits, obtained from Roche Diagnostics (Mannheim, Gwermany), were used to measure serum Apo A1 and Apo B at the Hitachi system (Roche Diagnostics).

3.5 Dietary assessment

Habitual dietary intake of the participants was assessed by the use of a FFQ (supplement C), developed by the Department of Nutrition at the University of Oslo (Oslo, Norway), and has been validated for several nutrients [119-121]. The FFQ was handed out to the patients on the day of recruitment, and were returned to the study center by mail, or collected one month later at the follow-up appointment. The FFQ was used to acquire information on the patient's habitual food intake during the recent year, and included a total of 169 food items. The food items were arranged into groups according to Norwegian food-consumption patterns. The portion sizes were given as household measures, such as pieces or slices. The options for frequency of consumption were per day, week or month, depending on the food items in the question. Average daily intake was used for the analyses. Daily energy and nutrient intake reported in the FFQ was estimated using a database and software system developed at the Institute for Nutrition Research at the University of Oslo (Kostberegningssystem, version 3.2; University of Oslo, Norway). The food database is mainly based on the official Norwegian Food Table (1995), which is continuously updated [121]. Dietary betaine intake was calculated using values from the United States Department of Agriculture (USDA) database for the choline content of common foods, release two [86].

3.6 Statistical analysis

To test for normality of variance among dietary data, Kolmogorov-Smirnov´s test and Shapiro-Wilk´s test was used. Baseline data were presented as medians (25^{th}, 75^{th} percentiles) for continuous variables, using the Tukey´s Hinges method, and n (%) for dichotomous variables. Significance value for trend was calculated by using unadjusted linear regression for continuous variables and unadjusted logistic regression for dichotomous variables. Correlation analyses were performed using Spearman´s rank correlation coefficient, expressed as r. Adjustments for age (continuous), gender (dichotomous), total daily EI (continuous) and statin use (dichotomous), were made through partial Spearman correlation analyses. Multiple linear regression analyses were used to study associations between quartiles of betaine intake and serum lipid levels in a multivariate method adjusted for age, gender, body mass index (BMI) (continuous), total EI, smoking (dichotomous), diabetes (dichotomous), and statin treatment. Furthermore, to check for non-linear associations analyses were repeated with each of the three upper quartiles of betaine intake represented as dummy variables in the models.

To further evaluate potential tail effects, the association of particular high betaine intake, i.e., the upper decile, was examined in the same models. Because folate status as well as statin treatment theoretically might influence the effect of betaine intake on lipid metabolism, we finally evaluated the potential effect modification by these two parameters. The analyses focusing on a particular high betaine intake were therefore repeated by dividing the population in two according to median serum folate levels or treatment with cholesterol lowering statins. All probability values were two-tailed, and P-values <0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS version 21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

4.0 Results

4.1 Baseline clinical characteristics

Clinical characteristics at baseline of 2026 participants by quartiles of dietary betaine intake are presented in **Table 2**. Median (25^{th}, 75^{th} percentile) age was 62 (55, 79) years and 80.4% of the participants were men. Median BMI was 26.5 (24.5, 28.7) kg/m^2. A previous acute MI was reported in 43.6%, percutaneous coronary intervention in 22.8% and coronary artery bypass graft surgery in 14.3% of the participants. A total of 31.0% were diagnosed with DM, and 47.8% received treatment for hypertension, while 29.8% were current smokers. A minority of the participants (10.1%) was considered to have an impaired LVEF. At coronary angiography 11.3% had non-significant CAD, while 28.5% had a single-, 27.3% double-, and 32.8% triple vessel disease. The majority of the participants received medical treatment with acetylsalicylic acid (83.9%), statins (79.2%) and β-blockers (75.4%).

Those with the highest intake of betaine were younger ($p<0.001$), more likely to be males ($p<0.001$), had a lower BMI ($p=0.04$), were less likely to be hypertensive ($p=0.002$), to have impaired LVEF ($p=0.03$), and to have non-significant CAD ($p=0.03$), and more likely to receive treatment with acetylsalicylic acid ($p=0.001$), compared to those with a low intake. Furthermore, higher intakes were associated with higher estimated glomerular filtration rate ($p<0.001$).

Table 2: Baseline clinical characteristics of participants with stable angina pectoris by quartiles of betaine intake (n=2026)[1]

	Quartiles of betaine intake				
	1	2	3	4	P for trend[2]
Betaine (mg)	86.1 (72.7, 96.7)[3]	119 (112, 127)	148 (142, 159)	198 (180, 226)	
Age (y)	65.0 (58.0, 72.0)	62.0 (56.0, 70.0)	61.0 (55.0, 68.0)	59.0 (53.0, 66.0)	<0.001
Gender; male [n (%)]	299 (59.1)	407 (80.6)	445 (87.4)	477 (94.3)	<0.001
Body mass index (kg/m^2)	27.0 (24.7, 29.0)	26.5 (24.7, 28.7)	26.2 (24.2, 28.7)	26.2 (24.3, 28.7)	0.04
Cardiovascular history [n (%)]					
Myocardial infarction	229 (45.2)	224 (44.3)	219 (43.0)	211 (41.7)	0.22
Percutaneous coronary intervention	131 (25.9)	120 (23.7)	112 (22.0)	98 (19.3)	0.89
Coronary artery bypass graft surgery	86 (17.0)	80 (15.8)	64 (12.6)	59 (11.6)	0.85
Risk factors [n (%)]					
Diabetes mellitus[4]	152 (0.30)	155 (0.31)	159 (0.31)	162 (0.32)	0.48
Hypertension[5]	276 (54.5)	231 (45.7)	238 (46.7)	223 (44.1)	0.002
Current smoker[6]	141 (27.8)	150 (29.7)	160 (31.4)	154 (30.4)	0.30
Extent of CAD [n (%)]					
LVEF< 50%	55 (10.8)	62 (12.3)	51 (10.0)	37 (7.3)	0.03
No ore non-significant CAD	76 (15.0)	53 (10.5)	38 (7.4)	58 (11.4)	0.03
One-vessel disease	136 (26.9)	143 (28.3)	152 (29.8)	147 (29.0)	0.37
Two-vessel disease	127 (25.1)	141 (27.9)	141 (27.7)	145 (28.6)	0.24
Three-vessel disease	166 (32.8)	167 (33.1)	178 (35.0)	154 (30.4)	0.58
Medication before baseline coronary angiography [n (%)]					
Acetylsalicylic acid	396 (78.2)	425 (84.1)	447 (87.8)	431 (85.2)	0.001
Statin treatment	396 (76.9)	402 (79.6)	413 (81.1)	393 (77.6)	0.98
Beta-blockers	384 (75.9)	379 (75.0)	376 (73.9)	389 (76.9)	0.84
Glucose homeostasis, inflammation and renal function					
Glucose (mmol/L)	5.6 (5.1, 6.6)	5.6 (5.1, 6.5)	5.6 (5.1, 6.6)	5.6 (5.1, 6.5)	0.75
HbA1c (%)	5.8 (5.1, 6.6)	5.8 (5.1, 6.5)	5.8 (5.1, 6.6)	5.9 (5.1, 6.6)	0.62
C-reactive protein (mg/L)	1.73 (0.87, 3.24)	1.77 (0.85, 3.48)	1.61 (0.83, 3.12)	1.51 (0.75, 2.81)	0.61
eGFR (mL/min)	89.0 (78.0, 97.0)	91.0 (81.0, 99.0)	93.0 (83.0, 101.0)	95.0 (86.0, 102.0)	<0.001

[1] n=506, 505, 509 and 506 in quartiles 1 – 4, respectively.

[2] Calculated using linear and logistic regression for continuous and dichotomous variables, respectively.

[3] Median (25th, 75th percentile) for all such values

[4] Based on self-report, HbA1c ≥ 6.5 %, fasting glucose level ≥ 7.0 mmol/L and nonfasting glucose level ≥11.1 mmol/L.

[5] Patients receiving medical treatment for hypertension.

[6] Based on self-report, stopped smoking < 1 month ago, and cotinine levels > 85 nmol/L.

4.2 Daily dietary intake

Daily dietary intakes among the 2026 participants are presented in **Table 3**. Median (25^{th}, 75^{th} percentile) dietary betaine intake in the cohort was 134 (104, 168) mg/d. Increasing intakes of betaine were associated with a higher total energy intake, and also with higher intakes of carbohydrate, dietary fiber ($p<0.001$ for both) and polyunsaturated fat (%TE) ($p=0.001$). A high betaine intake was however inversely associated with intake of saturated fat (%TE), monounsaturated fat (%TE) and protein (%TE) (all $p<0.001$).

Table 3: Daily dietary intake by quartiles of betaine intake (n=2026)[1]

	Quartiles of betaine intake				
	1	2	3	4	P for trend[2]
Energy (kJ)	6134 (5045, 7438)[3]	7890 (6876, 9041)	9099 (7978, 10347)	11032 (9764, 12895)	<0.001
Carbohydrates (%TE)[4]	49.7 (44.9, 54.0)	49.1 (45.3, 53.1)	49.8 (45.5, 53.9)	50.5 (46.4, 54.6)	<0.001
Dietary fiber (%TE)	49.7 (44.9, 54.0)	49.1 (45.3, 53.1)	49.8 (45.5, 53.9)	50.5 (46.4, 54.6)	<0.001
Total fat (%TE)	31.2 (27.3, 35.0)	32.1 (28.2, 35.7)	31.2 (28.0, 34.9)	30.7 (27.6, 34.4)	0.13
Saturated fat (%TE)	11.6 (9.9, 13.3)	11.7 (10.2, 13.3)	11.3 (9.6, 13.1)	11.1 (9.5, 12.7)	<0.001
Monounsaturated fat (%TE)	10.1 (8.7, 11.6)	10.4 (8.9, 11.6)	10.0 (8.9, 11.3)	9.9 (8.7, 11.0)	<0.001
Polyunsaturated fat (%TE)	6.4 (5.4, 8.0)	6.9 (5.7, 8.3)	6.8 (5.7, 8.3)	7.0 (5.9, 8.5)	0.001
Total proteins (%TE)	17.4 (15.6, 19.2)	16.7 (15.3, 18.4)	16.8 (15.2, 18.5)	16.2 (14.9, 17.8)	<0.001
Alcohol (%TE)	0.70 (<0.00, 2.80)	1.10 (<0.00, 2.80)	1.10 (0.10, 3.10)	1.10 (0.10, 2.80)	0.29

[1] n=506, 505, 509 and 506 for quartiles 1 – 4, respectively.

[2] Calculated using linear regression for continuous variables.

[3] Median (25^{th}, 75^{th} percentile) for all such values.

[4] Percent of total energy intake.

4.3 Baseline blood parameters

Circulating levels of parameters related to choline oxidation and lipid metabolism among the participants are presented in **Table 4**. Higher betaine intakes were associated with lower plasma tHcy (p=0.001), glycine (p=0.03) and folate (p<0.001), and with higher plasma betaine (p<0.001), betaine-to-choline ratio (p<0.001), and serum sarcosine (p<0.001). Furthermore, betaine intake was associated with lower serum total cholesterol (p=0.009), LDL-cholesterol (p=0.04), HDL-cholesterol (p<0.001), Apo B (p=0.049) and Apo A1 (p=0.001).

Table 4: Circulating levels of parameters related to choline and lipid metabolism of by quartiles of betaine intake (n=2026)[1]

	Quartiles of betaine intake				
	1	2	3	4	P for trend[2]
Parameters related to the choline and one-carbon metabolism					
Total homocysteine (μmol/L)	10.6 (8.7, 12.9)[3]	10.2 (8.7, 12.3)	10.3 (8.7, 12.1)	9.9 (8.5, 11.8)	0.001
Choline (μmol/L)	9.4 (8.1, 11.2)	9.6 (8.1, 11.3)	9.6 (8.3, 11.2)	9.5 (8.0, 11.4)	0.82
Betaine (μmol/L)	36.2 (29.8, 45.7)	37.8 (32.2, 46.9)	40.3 (33.3, 48.7)	41.4 (34.9, 49.8)	<0.001
Betaine-to-choline ratio	3.84 (3.16, 4.68)	4.00 (3.35, 4.70)	4.26 (3.50, 5.0)	4.37 (3.64, 5.2)	<0.001
Dimethyl glycine (μmol/L)	3.76 (3.09, 4.72)	3.98 (3.26, 4.91)	4.08 (3.40, 5.0)	4.03 (3.33, 4.74)	0.26
Sarcosine (μmol/L	1.39 (1.13, 1.80)	1.45 (1.14, 1.79)	1.53 (1.25, 1.94)	1.55 (1.27, 1.93)	<0.001
Serine (μmol/L	93.9 (80.8, 108)	93.1 (80.6, 104)	93.9 (83.2, 108)	93.5 (80.7, 107)	0.73
Glycine (μmol/L	200 (174, 239)	195 (173, 223)	198 (173, 229)	198 (176, 226)	0.03
Folate (nmol/L)	10.1 (7.2, 15.0)	10.0 (7.5, 15.2)	9.9 (7.14, 13.9)	9.9 (7.4, 13.6)	<0.001
Parameters related to lipid metabolism					
Total cholesterol (mmol/L)	5.0 (4.40, 5.8)	4.90 (4.30, 5.60)	4.80 (4.20, 5.5)	4.70 (4.10, 5.6)	0.01
LDL-cholesterol (mmol/L)	2.98 (2.43, 3.70)	2.88 (2.40, 3.60)	2.89 (2.84, 3.50)	2.80 (2.20, 3.50)	0.04
HDL-cholesterol (mmol/L)	1.28 (1.01, 1.50)	1.20 (1.00, 1.45)	1.20 (1.00, 1.40)	1.20 (1.00, 1.40)	<0.001
Triacylglycerol (mmol/L)	1.49 (1.10, 2.09)	1.55 (1.12, 2.27)	1.57 (1.10, 2.17)	1.55 (1.10, 2.28)	0.34
Apo B (g/L)	0.86 (0.71, 1.03)	0.85 (0.73, 1.01)	0.84 (0.73, 0.99)	0.83 (0.68, 1.02)	0.049
Apo A1 (g/L)	1.29 (1.11, 1.49)	1.25 (1.11, 1.43)	1.25 (1.11, 1.43)	1.26 (1.09, 1.41)	0.001

[1] n=506, 505, 509 and 506 in quartiles 1 – 4, respectively.

[2] Calculated using linear regression for continuous variables.

[3] Median (25^{th}, 75^{th} percentile) for all such values.

4.4 Dietary betaine intake in association with plasma choline metabolites

Spearman correlation analyses were used to explore the relationship between dietary betaine intake and circulating levels of metabolites along the choline oxidation pathway, including choline, betaine, tHcy, DMG, sarcosine, glycine and serine. In unadjusted analyses, betaine intake was positively associated with plasma betaine ($r=0.17$, $p<0.001$) and DMG ($r=0.06$, $p=0.006$) and with serum sarcosine ($r=0.12$, $p<0.001$) and inversely associated with plasma tHcy ($r=-0.09$, $p<0.001$). There were no associations between dietary betaine intake and concentrations of choline, glycine and serine in plasma. The association between betaine intake and plasma betaine was attenuated by adjusting for age, gender, total EI and statin use ($r=0.10$, $p<0.001$). Otherwise, none of the other associations were appreciably affected after multivariate adjustment (**Table 5).**

Table 5: Spearman correlations between dietary betaine intake and circulating levels of metabolites related to the choline oxidation pathway.

	Unadjusted		Adjusted[1]	
	Coefficient	P-value	Coefficient	P-value
Betaine	0.17	<0.001	0.10	<0.001
Choline	0.004	0.87	0.01	0.68
Total homocysteine	- 0.09	<0.001	- 0.09	<0.001
DMG	0.06	0.006	0.06	0.006
Sarcosine	0.12	<0.001	0.07	0.001
Glycine	- 0.02	0.45	0.03	0.21
Serine	0.002	0.93	0.03	0.23

[1] Adjusted for age, gender, total energy intake and statin use.

Because folate and betaine metabolisms converge at the point of Hcy in the one-carbon metabolism, serum folate status is believed to affect the flux of betaine through BHMT, and thereby influence the plasma betaine levels [122]. Spearman correlation analysis was thus used to investigate the association between betaine and folate in plasma. The results revealed a significant positive association in crude analysis ($r=0.15$, $p<0.001$), which was not attenuated after adjusting for age, gender, and statin use ($r=0.17$, $p<0.001$).

4.5 Dietary betaine intake in association with lipid related parameters in plasma

The relationships between reported dietary betaine intake and serum levels of parameters related to lipid metabolism, including total cholesterol, LDL-cholesterol, HDL-cholesterol, TGs, Apo A1 and Apo B were investigated by using multiple linear regression. We first evaluated a simple model adjusted for age and gender followed by a more extensive multivariate model. Because these models provided similar results we only present the results of the multivariate models (**Table 6**).

Table 6: Association between quartiles of betaine intake with lipid related parameters in serum assessed by multiple linear regression (n=2026).

	Coefficient[1]	Standard error	P-value
Total cholesterol			
Quartiles of betaine intake	- 0.03	0.30	0.24
Age (years)	- 0.12	0.003	<0.001
Male gender	- 0.29	0.06	<0.001
BMI (kg/m^2)	0.02	0.01	0.01
Energy intake (kJ/day)	<0.001	<0.001	0.61
Current Smoking[2]	0.04	0.05	0.50
Statins	- 1.29	0.06	<0.001
Diabetes[3]	- 0.12	0.05	0.02
LDL-cholesterol			
Quartiles of betaine intake	- 0.02	0.02	0.33
Age (years)	- 0.01	0.002	<0.001
Male gender	- 0.11	0.05	0.03
BMI (kg/m^2)	0.02	0.01	0.01
Energy intake (kJ/day)	<0.001	<0.001	0.26
Current Smoking	0.02	0.05	0.65
Statins	- 1.24	0.05	<0.001
Diabetes	- 0.18	0.04	<0.001
HDL-cholesterol			
Quartiles of betaine intake	- 0.004	0.01	0.61
Age (years)	0.004	0.001	<0.001
Male gender	- 0.22	0.02	<0.001
BMI (kg/m^2)	- 0.02	0.002	<0.001
Energy intake (kJ/day)	<0.001	<0.001	0.18
Current Smoking	- 0.04	0.02	0.02
Statins	- 0.003	0.02	0.87
Diabetes	- 0.02	0.02	0.18
Triacylglycerol			
Quartiles of betaine intake	- 0.02	0.03	0.48
Age (years)	- 0.02	0.003	<0.001
Male gender	0.14	0.07	0.04
BMI (kg/m^2)	0.07	0.01	<0.001
Energy intake (kJ/day)	<0.001	<0.001	0.52
Current Smoking	0.08	0.06	0.18
Statins	- 0.16	0.06	0.01
Diabetes	0.18	0.06	0.001

Apo A1			
Quartiles of betaine intake	- 0.002	0.01	0.79
Age (years)	0.002	0.001	<0.001
Male gender	- 0.15	0.01	<0.001
BMI (kg/m^2)	- 0.01	0.001	<0.001
Energy intake (kJ/day)	- 0.01	<0.001	0.31
Current Smoking	- 0.01	0.01	0.41
Statins	0.00	0.01	0.99
Diabetes	- 0.03	0.01	0.01
Apo B			
Quartiles of betaine intake	- 0.01	0.01	0.24
Age (years)	- 0.003	0.001	<0.001
Male gender	- 0.01	0.01	0.38
BMI (kg/m^2)	0.01	0.001	<0.001
Energy intake (kJ/day)	<0.001	<0.001	0.35
Current Smoking	0.02	0.01	0.09
Statins	- 0.25	0.01	<0.001
Diabetes	- 0.02	0.01	0.04

[1] Beta unstandardized coefficient

[2] Based on self-report, stopped smoking < 1 month ago, and cotinine levels >85 nmol/L.

[3] Based on self-report, HbA1c ≥6.5 %, fasting glucose level ≥7.0 mmol/L and nonfasting glucose level ≥11.1 mmol/L

Overall, no significant associations were revealed across quartiles of betaine intake with any of the parameters of lipid metabolism in the total study cohort. Because folate status as well as statin treatment theoretically might influence the effect of betaine intake on lipid metabolism, we finally evaluated the potential effect modification by these two parameters.

4.5.1 Dietary betaine intake in association with lipid related parameters in subgroups

Since serum folate status may influence plasma betaine levels, it may also influence the association between dietary betaine intake and lipid related parameters in plasma. We therefore performed multiple linear regression analysis in strata of below and above median serum folate (**Table 7**). Results revealed no significant associations in strata of below and above median serum folate.

Table 7: Association between quartiles of betaine intake with lipid related parameters in serum in patients with either low (<median) or high (≥median) serum folate concentrations assessed by multiple linear regression[1].

	Low serum folate[2]			High serum folate[3]		
	Coefficients[4]	Std. error	P value	Coefficients	Std. error	P value
Total cholesterol	- 0.04	0.04	0.38	- 0.04	0.04	0.37
LDL-cholesterol	- 0.02	0.04	0.66	- 0.03	0.03	0.32
HDL-cholesterol	- 0.02	0.01	0.20	0.02	0.01	0.86
Triacylglycerol	- 0.01	0.04	0.84	- 0.04	0.05	0.48
Apo A1	- 0.01	0.01	0.56	- 0.001	0.01	0.93
Apo B	- 0.01	0.01	0.30	- 0.01	0.01	0.54

[1] Adjusted for age, gender, BMI, total energy intake, diabetes, current smoking and statin treatment.
[2] Serum folate levels < median (9.98 nmol/L)
[3] Serum folate levels ≥ median (9.98 nmol/L)
[4] Beta unstandarized coefficients.

The majority (79.2%) of the participants in the present study received medical treatment with statins. Statins are lipid modifying drugs that lowers plasma total cholesterol levels by inhibition of hepatic cholesterol synthesis, and by enhanced uptake of plasma LDL-cholesterol into the liver [123], and may thus influence the association between betaine intake and lipid related parameters in plasma. Multiple linear regression analysis was therefore performed in strata of non-users and users of statins (**Table 8**).

Table 8: Association between quartiles of betaine intake and lipid related parameters in serum in patients without and with statin treatment assessed by multiple linear regression[1]

	No statin treatment			Statin treatment		
	Coefficients[2]	Std. error	P value	Coefficients	Std. error	P value
Total cholesterol	- 0.07	0.07	0.32	- 0.06	0.03	0.06
LDL-cholesterol	0.06	0.06	0.30	- 0.04	0.03	0.09
HDL-cholesterol	0.02	0.02	0.39	- 0.012	0.009	0.21
Triacylglycerol	- 0.04	0.06	0.51	- 0.016	0.04	0.64
Apo A1	0.02	0.02	0.25	- 0.008	0.01	0.29
Apo B	0.014	0.01	0.35	- 0.012	0.006	0.06

[1] Adjusted for age, gender, body mass index, total energy intake, diabetes and current smoking.
[2] Beta unstandarized coefficients.

Overall, there were no significant associations between dietary betaine intake and parameters related to lipid metabolism in plasma, neither in the total population, nor when stratifying by median folate status or when stratifying by statin treatment. A similar finding was revealed when using the second, third and fourth quartile of betaine intake as dummy variables.

4.5.2 Dietary betaine intake above the 90th percentile in association with lipid related parameters in the total cohort and in subgroups

Furthermore, in order to investigate whether there still may have been a tail effect illustrating an association between a particular high betaine intake and levels of lipid related parameters in plasma, we compared the upper decile of dietary betaine intake to the remaining cohort, where the cut off value was 207.76 mg/d, when performing multiple linear regression analysis (**Table 9**).

Table 9: The association between particular high betaine intake (≥ 90th percentile) with lipid related parameters (n=202)[1]

	Coefficient[3]	Standard error	P-value
Total cholesterol	- 0.11	0.09	0.22
LDL-cholesterol	- 0.14	0.07	0.052
HDL-cholesterol	**0.06**	**0.03**	**0.02**
Triacylglycerol	- 0.13	0.09	0.16
Apo A1	0.01	0.02	0.71
Apo B	**- 0.04**	**0.02**	**0.02**

[1] Adjusted for age, gender, body mass index, total energy intake, diebates, current smoking and statin treatment.
[2] Beta unstandardized coefficient.

Results revealed a significant positive association between particular high betaine intake and HDL-cholesterol (β=0.06, p=0.02) and an inverse association with Apo B (β=-0.04, p=0.02).

Based on these observations we performed similar multiple linear regression analyses in strata of below and above median serum folate (**Table 10**).

Table 10: The association between particular high betaine intake (≥ 90th percentile) with lipid related parameters in patients with either low (<median) or high (≥ median) serum folate assessed by multiple linear regression (n=202)[1]

	Low serum folate[1]			High serum folate[2]		
	Coefficients[3]	Std. error	P value	Coefficients	Std. error	P value
Total cholesterol	- 0.11	0.11	0.36	- 0.12	0.13	0.37
LDL-cholesterol	- 0.14	0.10	0.18	- 0.14	0.10	0.08
HDL-cholesterol	0.03	0.04	0.49	**0.09**	**0.04**	**0.02**
Triacylglycerol	- 0.05	0.11	0.65	- 0.23	0.16	0.15
Apo A1	- 0.01	0.03	0.72	0.02	0.03	0.50
Apo B	- 0.03	0.03	0.23	**- 0.05**	**0.03**	**0.03**

[1] Adjusted for age, gender, body mass index, total energy intake, diabetes, current smoking and statin treatment.
[2] Serum folate levels < median (9.98 nmol/L).
[3] Serum folate levels ≥ median (9.98 nmol/L).
[4] Beta unstandarized coefficients.

The results demonstrated no significant associations among those with low serum folate, while a significant positive association for a particular high betaine intake with HDL-cholesterol (β=0.06, p=0.02) and a significant inverse association with Apo B (β=-0.04, p=0.02) were observed among those with high serum folate.

We further evaluated the effect of particular high betaine intake and lipid levels according to statin treatment (**Table 11**).

Table 11: Association between particular high betaine intake (≥ 90th percentile) and lipid related parameters in patients without and with statin treatment assessed by multiple linear regression (n=202)[1].

	No statin treatment			Statin treatment		
	Coefficients[2]	Std. error	P value	Coefficients	Std. error	P value
Total cholesterol	- 0.02	0.20	0.93	- 0.14	0.10	0.14
LDL-cholesterol	- 0.02	0.18	0.91	**- 0.18**	**0.08**	**0.02**
HDL-cholesterol	0.10	0.06	0.11	0.05	0.03	0.06
Triacylglycerol	- 0.20	0.19	0.56	- 0.14	0.11	0.24
Apo A1	0.05	0.04	0.25	- 0.01	0.02	0.81
Apo B	- 0.02	0.04	0.73	**- 0.05**	**0.02**	**0.01**

[1] Adjusted for age, gender, body mass index, total energy intake, diabetes, current smoking, statin treatment.
[2] Beta unstandarized coefficients.

Results revealed no significant or tendency towards such an association between having a particular high dietary betaine intake and lipid parameters among those who were not treated with statins. However, significantly lower LDL-cholesterol (β=-0.18, p=0.02) and Apo B levels (β=-0.05, p=0.01) were observed among those receiving statins.

5.0 Discussion

Overall, we found weak, yet statistically significant associations between dietary betaine intake and plasma levels of some selected metabolites along the choline oxidation pathway, including positive associations with betaine, DMG and sarcosine and an inverse association with tHcy. We did not find any associations between quartiles of dietary betaine intake and lipid-related parameters, despite an association between intake and lipid parameters in unadjusted analyses. However, patients having a particular high betaine intake, corresponding to the upper decile, had significantly higher HDL-cholesterol and lower Apo B levels. This tail effect was only observed among those with a high folate status. In patients treated with statins, a particular high betaine intake was associated with both significantly lower LDL-cholesterol and Apo B levels.

5.1 Methodological discussion

5.1.1 Population and study design

The participants in the present study were part of a large and well-characterized cohort, consisting of 3090 randomized patients with angiographically verified CAD as the main inclusion criteria. Only patients examined due to suspected SAP were included in the current study, the majority receiving lipid lowering statins, and the results are therefore primarily valid for such patients. Clinical, anthropometric and dietary data were collected usually just after the angiographic procedure for each patient, providing an overview of the outcome and the associated characteristics of the patients available for assessment at this specific point in time [124]. This study is therefore cross-sectional [125]. The major advantage of a cross-sectional study design is that it generally is a fast and an un-expensive way to determine the amount of individuals with a disease and/or risk factors for the disease [124, 126], and also because it is useful to identify associations [127]. However, the limitation of cross-sectional studies are related to the fact that they are carried out at a one specific time-point, which give rise to the assumption that the associations may not exist outside this given point in time [126]. Thus, it is not possible to infer causality [125, 126].

5.1.2 Dietary assessment

Dietary intake can be assessed by using four basic methods, including dietary 24h recalls, food records, diet histories and FFQs, which all have their strengths and weaknesses [128]. In the present study, dietary intake of the subjects was assessed by use of a self-administered FFQ. This dietary assessment method is widely used in nutritional epidemiologic studies, and is designed for the assessment of an average diet for longer periods of time, such as weeks, months or years, rather than a diet lasting only a few days [129]. The quality of the FFQ depends on the responder's ability to correctly report their diet, and tends to be influenced by their present diet and their perception of what constitutes an appropriate diet [128]. Another limitation is that the subjects may not be able to accurately report the correct frequency of consumption and portion size, and errors related to this issue may occur [129]. These errors are part of information biases which may affect the accuracy, and hence the quality of the dietary assessment [130]. The FFQ used in the present study included 169 food items, and included important sources of betaine, such as bread, breakfast cereals, pasta and root vegetables, making it possible to estimate betaine intake. The FFQ has not been validated for betaine, but for a number of nutrients, foods and also energy intake [131-133]. Unfortunately, no good biomarkers for betaine are currently available, which is why we did not have the possibility to explore the validity of the calculated betaine intake estimates this in our study.

Some actions have been taken to improve the dietary data. Individuals with reported total daily EI regarded as unrealistic were excluded. Also, due to resource constraints, the current FFQs were not controlled for errors when handed in, and in order to somewhat minimize this issue participants reporting >1 blank page were excluded. Due to generally poor precision with FFQs, they are better suited for ranking or classifying individuals than using absolute values; hence, individuals were either ranked or divided into quartiles when performing statistical analyses. Another issue that may affect the accuracy of dietary intake assessment is the database used to calculate the nutrient intakes. However, betaine intake was estimated by using the USDA database for the choline content in common foods [86], where the values in the database has been evaluated and reported to be of good quality [86].

Furthermore, when comparing participants who responded with those who did not respond to the FFQ, there were certain differences. Non-responders were more likely to be women, to be current smokers, to have diabetes, reduced ejection fraction and higher serum

total cholesterol, Apo B and C-reactive protein, but less likely to have previously undergone a percutaneous coronary intervention [116, 134]. Because the characteristics of the non-responders were not taken into account in the current analyses, the differences between responders and non-responders may bias the results [125, 135, 136].

5.1.3 Biochemical analysis

The analyses of standard blood laboratory parameters were performed according to routine protocols at the two university hospitals in Western Norway. The one-carbon metabolites analyzed at Bevital by GC-MS/MS, including tHcy, sarcosine, glycine and serine, had a recovery between 79% and 99% [137], while the compounds analyzed by LC-MS/MS, including choline, betaine, tHcy and DMG had a recovery ranging from 75% to 123% [138]. To assess the assay validation, regression analyses were performed, which showed a linear relationship between area ratio and the concentration of the analytes.

5.1.4 Confounding

The general definition of the term confounding can be explained by referring to the association between two variables, a specific exposure and an outcome, which are influenced by a third variable or group of variables, known as the confounding variable, or the confounder, where the confounder are non-casually associated with the exposure and causally associated with the outcome [139-141]. In the present study, the exposure is dietary betaine intake and the outcome is the metabolites along the choline oxidation pathway and parameters related to lipid metabolism. As a consequence of confounding, the strength of the association between the exposure and the outcome may be over- or underestimated [139], and also lead to a change in the direction of the association [124]. Confounding may thus generate biased results, which can be reduced or eliminated by making the appropriate statistical adjustments. In nutritional epidemiology, age and gender are among some of the variables that are considered to be potentially confounders due to the fact that they are correlated with many other factors [140]. Furthermore, because total EI is correlated with intake of many nutrients it must be accounted for, especially when analyzing data that are not energy adjusted [129]. In addition, statin use may be a confounder due to its lowering effect on serum total cholesterol levels [123, 142]. Age, gender, total EI and statin use were thus adjusted for when performing partial Spearman´s rank correlations. Additional factors that

may have a confounding effect on the association between betaine intake and lipid related parameters in plasma are BMI, diabetes and smoking. Hence, these factors were also adjusted for when performing multiple linear regression.

5.1.5 Energy adjustment

In the present study, adjustment for total EI was made by using the standard multivariate model [129]. When performing Spearman's rank correlations and multiple linear regressions, both betaine and total EI was included simultaneously as independent variables. However, there is no general agreement on which approach that accomplishes this most efficiently, and several methods currently exist. One alternative approach may be to use the residual method, where the nutrient intake of each individual are plotted against their total EI in a linear regression model to produce residuals to which a constant is usually added [143]. This or other methods could be considered in further analyses.

5.1.6 Statistical analysis

Reported dietary betaine intake among the current participants was not normally distributed. Therefore, to explore the associations between dietary intake of betaine and plasma metabolites along the choline oxidation pathway Spearman´s rank correlation was used. Spearman rank correlation is a non-parametric statistical method which do not require a normal distribution [144], and was suitable in the present study where obtained data from all participants were ranked prior to analysis [145]. This method measures the strength and direction of the association between two variables, while a partial Spearman rank correlation allows an adjustment for other covariates [146]. In addition, the Spearman rank correlation is also less sensitive to outliers. Although the Spearman correlation coefficient do not infer causality, it is useful with regard to discussing probable mechanisms based on the strength and direction of the observed association [145, 147].

Multiple linear regression was applied in order to investigate possible associations between dietary betaine intake and parameters related to lipid metabolism. Multiple linear regression is a parametric statistical method, which is based on correlation, and is used to explore the association between one continuous dependent variable and a number of independent variables [146]. In the present study lipid related parameters in plasma were the dependent variables, while betaine intake in addition to age, gender, total EI, BMI, diabetes,

current smoking, and statin treatment were the independent variables. When performing a multiple linear regression analysis, all the independent variables are included simultaneously, where each of their predictive power is being assessed, and allows the investigator to sort out the many different features of risk factors and explore how they affect the outcome [141]. In addition, stratified analyses may be useful in order to assess effect modification [148, 149]. Since folate status is believed to affect plasma betaine levels, and statin treatment is known to influence lipid metabolism, the analyses were performed separately in those with serum folate below and above median, as well as in non-users and users of statins. However, the statistical package used did not allow to test for significant interaction, and the results obtained by stratification are therefor only exploratory and should motivate further investigations

5.2 Discussion of results

5.2.1 Betaine intake and plasma metabolites of the choline oxidation pathway

In the current cohort median (25^{th}, 75^{th} percentile) dietary betaine intake was 134 (104, 168) mg/d, which is similar compared to the estimated intakes in large cohorts in the US and Europe, where intakes have been reported to generally range between 100 and 300 mg/d [87-91]. Most studies on this subject have focused on studying the effect of betaine supplementation, and due to a lack of food-composition databases in the previous years, only fewer studies have investigated the effect of a habitual dietary intake of betaine. Several studies have demonstrated consumption of betaine supplements to lead to an immediate increase in plasma betaine concentrations and a decrease in plasma tHcy concentrations, occurring in a dose dependent manner [74, 150-157]. Similar results have been obtained after ingestion of a betaine-enriched meal [158, 159]. A significant increase in plasma DMG has also been reported after betaine consumption in high doses [156, 157, 159]. In agreement with the current results, previous studies have also revealed a significant inverse association of dietary betaine with plasma tHcy [88, 91, 160].

The current findings are, however, not directly comparable with studies using supplements and betaine-enriched diets, which have a substantially higher betaine content (generally 1-6 g) compared to that of an average diet. Thus, it appears that a betaine intake, which exceeds that of an average daily diet, produces stronger associations with plasma betaine, tHcy and DMG.

5.2.2 Betaine intake and plasma levels of lipid related parameters

Few studies have investigated the association of dietary betaine intake and serum levels of lipid related parameters, and the reported effects of dietary and supplementary betaine intake on lipid metabolism from previous findings during the recent years are conflicting. The current findings are in agreement with findings from two cross-sectional studies among subjects with ACS attending a lipid clinic [161] and a large cohort of middle aged and elderly subjects [162], where plasma betaine was inversely associated with non-HDL-cholesterol and TG concentrations, and positively associated with HDL-cholesterol concentrations. However, in a study among healthy subjects [106], and a study among patients with chronic renal failure [163], betaine supplementation was associated with elevated LDL-cholesterol and TG concentrations, whereas in a study among obese subjects [151], and two studies among healthy subjects [156, 159] no significant associations were revealed.

5.3 Possible mechanisms

5.3.1 Betaine intake and plasma metabolites of the choline oxidation pathway

Dietary betaine intake was weakly, yet significantly associated with plasma one-carbon metabolites. Betaine is rapidly removed from plasma by being distributed into most tissues, mainly the liver and kidneys, where it is needed as an osmolyte or methyl donor [152]. Both the transport of betaine into tissues through the betaine transporter BGT1, and the metabolism of betaine by BHMT is osmoregulated [78, 103], ensuring betaine to be under strict metabolic control, which gives rise to inter-individual variability in serum betaine [157].

The observed significant associations with DMG and tHcy in plasma is in accordance with betaine being eliminated in the liver and kidney by betaine-dependent remethylation of Hcy into methionine, as it is metabolized to DMG, by BHMT [155, 158]. Furthermore, the transmethylation pathways of which folate and betaine enters in the one-carbon metabolism converges at the point of Hcy [75]. Plasma betaine levels have been reported to increase with folate supplementation, which may be a result of BHMT being

inhibited by 5-mTHF [73]. Moreover, among subjects with low folate status, plasma betaine has been reported to be a determinant with an increase in tHcy concentrations [122]. This suggests that the 2 transmethylation pathways are interrelated, and it may thus be hypothesized that lower circulating levels of folate increases the need for betaine as a methyl donor, while higher circulating levels of folate has a betaine sparing effect [73, 164]. Hence, we were interested in looking at the association between folate and betaine in plasma in the current study population, where a significant positive association was observed. Based on this observation, and the observed weak, although significant correlations between betaine intake and plasma one-carbon metabolites, it may be assumed that a high folate status leads to a lower activity of BHMT, which reduces the flow of betaine through the transmethylation pathway in the one-carbon metabolism. This is plausible due to the fact that folate-dependent remethylation occurs in most tissues, while betaine-dependent remethylatin is restricted to the liver and kidney [150]. Furthermore, adjusting for age, gender, total EI and statin use in the present study attenuated the association between betaine intake and plasma betaine, but did otherwise not appreciably affect the other associations.

5.3.2 Betaine intake and plasma levels of lipid related parameters

In the current cohort, a higher betaine intake was associated with lower HDL-, LDL- and total cholesterol levels, and lower Apo A1 and Apo B levels, when performing unadjusted linear regressions. Furthermore, when performing adjusted multiple linear regression analyses, no significant associations were observed between quartiles of betaine intake and lipid related parameters, neither in the total study population, nor in those with serum folate below and above median, or in non-users and users of statins. However, among those in the upper decile of betaine intake significantly higher HDL-cholesterol and lower Apo B levels were observed, when compared with the rest of the population. Moreover, those having a betaine intake corresponding to the upper decile had significantly higher HDL-cholesterol and lower Apo B levels. Moreover, in the stratified analyses, those with a betaine intake in the upper decile and who also had high serum folate had significantly higher HDL-cholesterol and lower Apo B levels, while those using statins had lower LDL-cholesterol and Apo B levels.

Results from previous studies with betaine supplements differ from those in the current study. High doses of betaine intake have been associated with increased LDL-cholesterol and TG levels among healthy subjects [106] and among subjects with chronic

renal failure [163]. In the present study, among patients with SAP on conventional medical treatment, a particular high dietary intake of betaine was associated with higher HDL-cholesterol and lower Apo B levels. The flux of betaine through BHMT in the transmethylation pathway is believed to be enhanced when betaine is increasingly available [157], which also have been previously demonstrated in animals [165-167]. It may thus be speculated that high doses of betaine intake affects the lipid metabolism differently compared with habitual dietary betaine intake. A possible mechanism linking betaine to the hepatic lipid metabolism have previously been hypothesized to be through enhanced methionine synthesis and the following increase in the SAM/SAH ratio, which ultimately leads to increased VLDL synthesis and secretion [95, 107].

The association between a particular high betaine intake and lipid related parameters among those with serum folate above median is interesting. A low folate status has been associated with accumulation of lipids in the liver, while less is known about the effect of a high folate status [168]. Supplementation with folic acid did not affect the lipid metabolism in a study among healthy subjects [106]. However, middle aged and elderly men and women had increased plasma betaine levels after receiving folic acid supplements [73], which suggests that folate and betaine metabolism may be interrelated. Because both betaine and folate is achieved from foods such as grains, cereals and vegetables [82-84, 169], a high dietary betaine intake and a high folate status may simply be an indicator of an overall healthy diet, which in general is associated with a favorable lipid profile [170, 171], including higher HDL-cholesterol levels [172].

Furthermore, other mechanisms might also be involved. 5-mTHF has been reported to reduce the flux through BHMT [73] and inhibit GNMT [70], two enzymes believed to be involved in lipid metabolism. First, the transcription of BHMT has been reported to be associated with co-transcription of Apo B [115], the main apolipoprotein involved in the VLDL synthesis in the liver. Second, the flux through BHMT may be involved in the transcription of nuclear transcription factor peroxisome proliferator activator receptor alpha [173-175], which is an important regulator in lipid metabolism [176]. Third, GNMT activity is associated with co-activity of the Nieman Pick CII like protein 2, which is involved in the transport of cholesterol from the lysosomes to be incorporated into VLDL or HDL particles [177].

Previously, circulating betaine has been inversely associated with LDL-cholesterol and TG levels and positively associated with HDL-cholesterol among subjects with ACS attending a lipid clinic and receiving treatment with statins [161]. Betaine intake was inversely associated with circulating LDL-cholesterol and Apo B levels among statin users having a particular high betaine intake in the current study. Unpublished data from WENBIT shows that statin treatment is associated with higher circulating betaine. This indicates that statin treatment might influence betaine synthesis or metabolism and this hypothesis should be explored in future studies.

The current observations indicate a change in how lipid metabolism is regulated depending on both folate status or statin treatment. However, these analyses have not been tested for interaction, and hence, it can not be determined with certainty whether the relationship between betaine intake and lipid related parameters in plasma are significantly different in strata of serum folate status and statin treatment.

6.0 Conclusion

In this cross-sectional study among 2026 patients with angiographically verified SAP, there were weak, yet statistically significant associations of habitual dietary intake of betaine with plasma metabolites of the choline oxidation pathway, including betaine, tHcy, DMG and sarcosine. Those in the upper decile of betaine intake with a high folate status had significantly higher plasma HDL-cholesterol and lower Apo B levels, while those using statins had significantly lower LDL-cholesterol and Apo B levels.

7.0 Future perspectives

The current observations of the association between a particular high betaine intake and lipid related parameters in plasma were made in patients with SAP. Further research should thus investigate these associations can be reproduced in other cohorts or subgroups in order to achieve a broader understanding on the effects of habitual dietary betaine intake on circulating levels of lipid related parameters. One appropriate way to study these associations into more detail is to perform randomized studies where eg. participants receive a betaine diet, compared to a control diet. Furthermore, due to challenges of measuring dietary intake, which may further bias the results, future studies should explore possible biomarkers for dietary intake of betaine.

Betaine has been hypothesized to affect the hepatic lipid metabolism through mechanisms that ultimately lead to increased VLDL synthesis and secretion. However, this was not observed in the present study. Such mechanisms may be studied by using cell culture or animal models, followed by measuring metabolites and lipids, as well as genes and/or enzymatic activities.

The association between betaine and lipid metabolism have also been suggested to be linked to the activation of the peroxisome-proliferator activated receptor alpha (PPARα). PPARα is a nuclear receptor protein, located mainly in the liver, kidney, heart and skeletal muscles, regulating the expression of several genes, including many involved in fatty acid beta-oxidation, and thus functions as a major regulator of energy homeostasis. In a study conducted in Apo E deficient mice, supplementation of betaine reduced hepatic TG content, resulting from reversed hypermethylation of the PPARα promoter. Due to limited studies on the association between betaine and PPARα, further research on this topic may be useful in order to reveal possible underlying mechanisms between betaine and hepatic lipid metabolism.

Betaine is considered to be an important modulator of the one-carbon metabolism, by serving as a methyl donor in a transmethylation pathway, where the first step along this pathway is catalyzed by BHMT. The availability of betaine is believed to affect the flow through BHMT. How to accurately measure this metabolic flow should thus be interesting to investigate in future research.

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HVA SPISER DU?

I dette skjemaet spør vi om dine spisevaner slik de **vanligvis** er. Vi er klar over at kostholdet varierer fra dag til dag. Prøv derfor så godt du kan å gi et **"gjennomsnitt"** av dine spisevaner. Ha det siste året i tankene når du fyller ut skjemaet. Der du er usikker, anslå svaret.

Skjemaet skal leses av en maskin, og derfor er det viktig at du setter et tydelig kryss i avmerket rute.

Riktig markering er slik: 

Bruk helst bløt blyant. Feil kan da rettes med viskelær.
Kulepenn og svart tusjpenn kan også brukes.

Av hensyn til den maskinelle lesingen pass på at arkene ikke blir brettet.

Alle svar vil bli behandlet strengt fortrolig.

EKSEMPEL PÅ UTFYLLING AV SPØRSMÅL 1.

Kari Nordmann spiser daglig 5 skiver brød og ett knekkebrød. Hun spiser vanligvis kneippbrød, men i helgene blir det en del loff. I tillegg spiser hun ett knekkebrød hver dag. Hun fyller ut første spørsmål slik:

1. HVOR MYE BRØD PLEIER DU Å SPISE?

Legg sammen det du bruker til alle måltider i løpet av en dag.

(1/2 rundstykke = 1 skive, 1 baguett = 5 skiver, 1 ciabatta = 4 skiver)

	Antall skiver pr. dag												
	0	1/2	1	2	3	4	5	6	7	8	9	10	11	12+
Fint brød (loff, baguetter, fine rundstykker o.l.)	☐	☐	☒	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mellomgrovt brød (lys helkorn, lys kneipp, lyst hj.bakt o.l.)	☐	☐	☐	☐	☐	☒	☐	☐	☐	☐	☐	☐	☐	☐
Grovt brød (fiberkneipp, mørk kneipp, mørkt hj.bakt o.l.)	☒	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Knekkebrød (kavring, grov skonrok o.l.)	☐	☐	☒	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Sum skiver pr. dag = 6

Antall skiver pr. uke: 6 x 7 = 42 Tallet brukes i spørsmål 5.

1. HVOR MYE BRØD PLEIER DU Å SPISE?

Legg sammen det du bruker til alle måltider i løpet av en dag.

(1/2 rundstykke = 1 skive, 1 baguett = 5 skiver, 1 ciabatta = 4 skiver)

	Antall skiver pr. dag												
	0	1/2	1	2	3	4	5	6	7	8	9	10	11	12+
Fint brød (loff, baguetter, fine rundstykker o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mellomgrovt brød (lys helkorn, lys kneipp, lyst hj.bakt o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Grovt brød (fiberkneipp, mørk kneipp, mørkt hj.bakt o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Knekkebrød (kavring, grov skonrok o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Sum skiver pr. dag = ____

Antall skiver pr. uke: ____ x 7 = ____. Tallet brukes i spørsmål 5.

2. HVA PLEIER DU Å SMØRE PÅ BRØDET?

Merk av både for hverdag og helg, selv om du bruker det samme.

Hverdager		Lørdager, søndager
☐	Bruker ikke	☐
☐	Smør (meierismør)	☐
☐	Bremykt, Smøregod	☐
☐	Brelett	☐
☐	Soft, soyamargarin (pakke, beger)	☐
☐	Solsikke	☐
☐	Oliven	☐
☐	Vita	☐
☐	Olivero	☐
☐	Omega	☐
☐	Soft light	☐
☐	Vita lett	☐
☐	Annen margarin	☐

3. OM DU BRUKER FETT PÅ BRØD, HVOR MYE BRUKER DU?

En porsjonspakning på 12 g rekker til antall skiver

1 ☐

2 ☐

3 ☐

4 ☐

5 ☐

4. MELK SOM DRIKK

(1 glass = 1,5 dl)

	Drikker sjelden/ ikke	Antall glass pr. dag								
		1/2	1	2	3	4	5	6	7	8+
Helmelk, søt, sur	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lettmelk, søt, sur	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lettmelk, ekstra lett	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Skummet melk, søt, sur	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

5. PÅLEGGSSORTER

Bruk sum skiver pr. uke fra spørsmål 1.

Til antall skiver pr. uke

	0	1/2	1	2-3	4-5	6-7	8-14	15-21	22-28	29-35	36+
Brun ost, prim	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hvit ost, helfet, 27% fett (Jarlsberg, Norvegia o.l., smøreost; eske, tube)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hvit ost, halvfet, 16% fett (Jarlsberg, Norvegia o.l., smøreost; eske, tube)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ost med mer enn 27% fett (kremoster, Normanna, Ridderost)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1/2	1	2-3	4-5	6-7	8-14	15-21	22-28	29-35	36+
Leverpostei, vanlig	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Leverpostei, mager	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Servelat, vanlig	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lett servelat, kalverull, kokt skinke, okserull o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salt pølse, spekepølse (fårepølse, salami o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1/2	1	2-3	4-5	6-7	8-14	15-21	22-28	29-35	36+
Kaviar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Makrell i tomat, røkt makrell	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sardiner, sursild, ansjos o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Laks, ørret	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Reker, krabbe	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1/2	1	2-3	4-5	6-7	8-14	15-21	22-28	29-35	36+
Syltetøy, marmelade, frysetøy	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Honning, sirup, sjokolade-, nøttepålegg	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1/2	1	2-3	4-5	6-7	8-14	15-21	22-28	29-35	36+
Grønnsaker som pålegg (agurk, tomat o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Frukt som pålegg (banan, eple o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salater med majones	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Majones på smørbrød	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

6. EGG

Antall pr. uke

	0	Mindre enn 1	1	2	3-4	5-6	7	8+
(kokt, stekt, eggerøre, omelett)	☐	☐	☐	☐	☐	☐	☐	☐

7. FROKOSTGRYN, GRØT OG YOGHURT

Svar enten pr. måned **eller** pr. uke. <1 betyr sjeldnere enn 1 gang.

	Gang pr. måned					Gang pr. uke						Mengde pr. gang				
	0	<1	1	2	3	1	2-3	4-5	6-7	8+		1	1 1/2	2	3+
Havregryn, kornblandinger (4-korn, usøtet müsli o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐
Cornflakes, puffet ris, havrenøtter o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐
												1-2	3-4	5-6	7+
Havregrøt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐
												1	2	3-4	5+
Sukker til frokostgryn, grøt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ts)	☐	☐	☐	☐
												1/2	1	1 1/2	2+
Yoghurt, naturell, frukt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(beger)	☐	☐	☐	☐
Lettyoghurt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(beger)	1/2 ☐	1 ☐	1 1/2 ☐	2+ ☐
Go´morgen yoghurt inkl. müsli	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(beger)	1/2 ☐	1 ☐	1 1/2 ☐	2+ ☐
												3/4	1	2	3+
Melk søt, sur på gryn, grøt og dessert	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐

8. KAFFE OG TE

(1 kopp kaffe = 1,2 dl 1 kopp te = 2 dl)

	Drikker ikke/ikke daglig	Antall kopper pr. dag 1/2	1	2	3-4	5-6	7-8	9-10	11+
Kaffe, kokt	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kaffe, traktet, filter	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kaffe, pulver (instant)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kaffe, koffeinfri	☐	☐	☐	☐	☐	☐	☐	☐	☐
Te	☐	☐	☐	☐	☐	☐	☐	☐	☐
Nypete, urtete	☐	☐	☐	☐	☐	☐	☐	☐	☐

	Antall teskjeer eller biter pr. kopp 0	1/2	1	2	3	4+
Sukker til kaffe	☐	☐	☐	☐	☐	☐
Sukker til te	☐	☐	☐	☐	☐	☐
Kunstig søtstoff til kaffe eller te	☐	☐	☐	☐	☐	☐
Fløte til kaffe	☐	☐	☐	☐	☐	☐

9. ANDRE DRIKKER?

Svar enten pr. måned **eller** pr. uke. < 1 betyr sjeldnere enn 1 gang.
Merk at porsjonsenhetene er forskjellige. 1/3 liter tilsvarer en halvflaske øl og 2/3 liter tilsvarer en helflaske.

	Gang pr. måned					Gang pr. uke						Mengde pr. gang					
	0	<1	1	2	3	1	2-3	4-5	6-7	8+		1/2	1	2	3	4	5+
Vann	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	☐	☐	☐	☐	☐	☐
Appelsinjuice	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	☐	☐	☐	☐	☐	☐
Annen juice, most, nektar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	☐	☐	☐	☐	☐	☐
Saft, solbærsirup m. sukker	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	☐	☐	☐	☐	☐	☐
Saft, kunstig søtet	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	☐	☐	☐	☐	☐	☐
Brus, Cola, Solo o.l., med sukker	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	☐	☐	☐	☐	☐	☐
Brus, Cola, Solo o.l., kunstig søtet	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	☐	☐	☐	☐	☐	☐
Farris, Selters, Soda o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	☐	☐	☐	☐	☐	☐
Alkoholfritt øl, vørterøl, lettøl	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	☐	☐	☐	☐	☐	☐
Pilsnerøl	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	☐	☐	☐	☐	☐	☐
Vin	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	☐	☐	☐	☐	☐	☐
Brennevin, likør	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(1 dram = 4 cl)	☐	☐	☐	☐	☐	☐

	1/2	1	2	3	4	5+
Vann	☐	☐	☐	☐	☐	☐
Appelsinjuice	☐	☐	☐	☐	☐	☐
Annen juice, most, nektar	☐	☐	☐	☐	☐	☐
Saft, solbærsirup m. sukker	☐	☐	☐	☐	☐	☐
Saft, kunstig søtet	☐	☐	☐	☐	☐	☐
Brus, Cola, Solo o.l., med sukker	1/4 ☐	1/3 ☐	1/2 ☐	2/3 ☐	1 ☐	11/2+ ☐
Brus, Cola, Solo o.l., kunstig søtet	1/4 ☐	1/3 ☐	1/2 ☐	2/3 ☐	1 ☐	11/2+ ☐
Farris, Selters, Soda o.l.	1/4 ☐	1/3 ☐	1/2 ☐	2/3 ☐	1 ☐	11/2+ ☐
Alkoholfritt øl, vørterøl, lettøl	1/4 ☐	1/3 ☐	1/2 ☐	2/3 ☐	1 ☐	11/2+ ☐
Pilsnerøl	1/4 ☐	1/3 ☐	1/2 ☐	2/3 ☐	1 ☐	11/2+ ☐
Vin	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6+ ☐
Brennevin, likør	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6+ ☐

10. MIDDAGSRETTER

Vi spør både om middagsmåltidene og det du spiser til andre måltider. Tell til slutt sammen antall retter du har merket for og se om summen virker sannsynlig.
En "dl" tilsvarer omtrent mengden i en suppeøse. Med "ss" menes en spiseskje.

	Gang pr. måned										Mengde pr. gang					
	0	<1	1	2	3	4	5-6	7-8	9+		1/2	2/3	1	11/2	2+	
Kjøttpølse, medisterpølse	☐	☐	☐	☐	☐	☐	☐	☐	☐	(kjøttpølse)	☐	☐	☐	☐	☐	
Hamburger, karbonader o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐	
Grill- og wienerpølse	☐	☐	☐	☐	☐	☐	☐	☐	☐	(pølse)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐	
Hamburger-, pølsebrød, lomper	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐	
Kjøttkaker, medisterkaker, kjøttpudding	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐	
Kjøttdeigretter (saus eller gryte med kjøttdeig, lasagne o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐	
Taco (med kjøtt og salat)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐	
Pastaretter	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐	

	Gang pr. måned										Mengde pr. gang				
	0	<1	1	2	3	4	5-6	7-8	9+		1/8	1/4	1/2	3/4	1+
Pizza (500-600 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(pizza)	☐	☐	☐	☐	☐
											1/2	1	1 1/2	2	2 1/2+
Biff (alle typer kjøtt)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐	☐
											1/2	1	1 1/2	2	2 1/2+
Koteletter (lam, okse, svin)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐	☐
											1-2	3-4	5-6	7-8	9+
Stek (lam, okse, svin)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(skive)	☐	☐	☐	☐	☐
											1-2	3-4	5-6	7-8	9+
Stek (elg, hjort, reinsdyr o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(skive)	☐	☐	☐	☐	☐
Gryterett med helt kjøtt, frikassé, fårikål o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Lapskaus, suppelapskaus, betasuppe	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
											1-2	3-4	5-6	7-8	9+
Bacon, stekt flesk	☐	☐	☐	☐	☐	☐	☐	☐	☐	(skive)	☐	☐	☐	☐	☐
											1/4	1/3	1/2	3/4	1+
Kylling, høne	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐	☐
											1-2	3-4	5-6	7-8	9+
Leverretter	☐	☐	☐	☐	☐	☐	☐	☐	☐	(skive)	☐	☐	☐	☐	☐
	0	<1	1	2	3	4	5-6	7-8	9+		1	2	3	4	5+
Fiskekaker, fiskepudding, fiskeboller	☐	☐	☐	☐	☐	☐	☐	☐	☐	(kake)	☐	☐	☐	☐	☐
											1-2	3-4	5-6	7-9	10+
Fiskepinner	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐	☐
											1	2	3	4	5+
Torsk, sei, hyse (kokt)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐	☐
											1	2	3	4	5+
Torsk, sei, hyse (stekt, panert)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐	☐
											1	2	3	4	5+
Sild (fersk, speket, røkt)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(filet)	☐	☐	☐	☐	☐
											1/2	1	1 1/2	2	3+
Makrell (fersk, røkt)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(filet)	☐	☐	☐	☐	☐
											1	2	3	4	5+
Laks, ørret (sjø, oppdrett)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(skive)	☐	☐	☐	☐	☐
Fiskegryte, -grateng, suppe med fisk	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
											1	2	3	4	5+
Reker, krabbe	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl, renset)	☐	☐	☐	☐	☐
	0	<1	1	2	3	4	5-6	7-8	9+						
Risgrøt, annen melkegrøt	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
											1-2	3-4	5-6	7-8	9+
Pannekaker	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐	☐
Suppe (tomat, blomkål, ertesuppe o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Vegetarrett, vegetarpizza grønnsakgrateng, -pai	☐	☐	☐	☐	☐	☐	☐	☐	☐	(bit/dl)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
	0	<1	1	2	3	4	5-6	7-8	9+		1/2	1	1 1/2	2	2 1/2+
Brun/hvit saus	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐	☐
Smeltet margarin, smør til fisk	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
											1	2	3	4	5+
Bearnaisesaus o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	☐	☐	☐	☐	☐
											1	2	3	4	5+
Majones, remulade	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	☐	☐	☐	☐	☐
											1	2	3	4	5+
Ketchup	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	☐	☐	☐	☐	☐

11. POTETER, RIS, SPAGHETTI, GRØNNSAKER

Svar enten pr. måned **eller** pr. uke. <1 betyr sjeldnere enn 1 gang.
Disse spørsmålene dreier seg først og fremst om tilbehør til middagsretter, men spiser du for eksempel en rå gulrot eller salat til lunsj, skal det tas med her.

	Gang pr. måned					Gang pr. uke						Mengde pr. gang				
	0	<1	1	2	3	1	2-3	4-5	6-7	8+		1	2	3	4	5+
Poteter, kokte	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐	☐
Pommes frites, stekte poteter	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐	☐
Potetmos, -stuing, gratinerte poteter	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐	☐
Ris	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Spaghetti, makaroni, pasta	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Gulrot	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1/2 ☐	1 ☐	1 1/2 ☐	2 ☐	3+ ☐
Hodekål	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(skalk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Kålrot	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(skive)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Blomkål	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(bukett)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Brokkoli	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(bukett)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Rosenkål	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Grønnkål	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Løk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Spinat, andre bladgrønns.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Sopp	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Avocado	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1/4 ☐	1/2 ☐	3/4 ☐	1 ☐	1 1/4 + ☐
Paprika	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(strimmel)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Tomat	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1/2 ☐	1 ☐	1 1/2 ☐	2 ☐	3+ ☐
Tomatbønner, bønner/linser	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Mais	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Erter, frosne grønnsak-blandinger	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Salatblandinger	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Dressing	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	1/2 ☐	1 ☐	2 ☐	3 ☐	4+ ☐
Rømme	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	1/2 ☐	1 ☐	2 ☐	3 ☐	4+ ☐

Hvor mange ganger om dagen spiser du vanligvis grønnsaker utenom grønnsakene du spiser til middag?

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5+ ☐

12. TYPE FETT TIL MATLAGING

Smør/margarin

- ☐ Smør (meierismør)
- ☐ Bremykt
- ☐ Melange, Per
- ☐ Soft-, soyamargarin (pakke, beger)
- ☐ Solsikke
- ☐ Oliven
- ☐ Annen margarin

Oljer

- ☐ Olivenolje
- ☐ Soyaolje
- ☐ Maisolje
- ☐ Solsikkeolje
- ☐ Valnøttolje
- ☐ Andre oljer

13. FRUKT

Svar enten pr. måned **eller** pr. uke. < 1 betyr sjeldnere enn 1 gang.

	Gang pr. måned					Gang pr. uke						Mengde pr. gang			
	0	<1	1	2	3	1	2-3	4-5	6-7	8+		1/2	1	2	3+
Eple	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐
Appelsin, mandarin, grapefrukt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐
Banan	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐
Druer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(klase)	☐	☐	☐	☐
Eksotisk frukt (kiwi, mango)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐
Annen frukt (fersken, pære m.v.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐
Jordbær, bringebær (friske, frosne)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐
Blåbær	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐
Multer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐

Hvor mange frukter spiser du vanligvis pr. dag?

0	1	2	3	4	5	6	7	8	9+
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

14. DESSERT, KAKER, GODTERI

Svar enten pr. måned **eller** pr. uke. < 1 betyr sjeldnere enn 1 gang.

	Gang pr. måned					Gang pr. uke						Mengde pr. gang			
	0	<1	1	2	3	1	2-3	4-5	6-7	8+		1/2	1	2	3+
Hermetisk frukt, fruktgrøt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐
Puddinger (sjokolade, karamell o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4+ ☐
Is (1 dl = 1 pinne = 1 kremmerhus)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4+ ☐
Boller, julekake, kringle	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4+ ☐
Skolebrød, skillingsbolle	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4+ ☐
Wienerbrød, -kringle o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4+ ☐
Smultring, formkake	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4+ ☐
Vafler	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(plate)	1/2 ☐	1 ☐	2 ☐	3+ ☐
Sjokoladekake, bløtkake, annen fylt kake	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1/2 ☐	1 ☐	2 ☐	3+ ☐
Søt kjeks, kakekjeks (Cookies, Bixit, Hob Nobs)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1-2 ☐	3-4 ☐	5-6 ☐	7+ ☐
Sjokolade (60 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(plate)	1/2 ☐	1 ☐	2 ☐	3+ ☐
Drops, lakris, seigmenn o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1-2 ☐	3-4 ☐	5-6 ☐	7+ ☐
Smågodt (1 hg = 100g)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(hg)	1/2 ☐	3/4 ☐	1 ☐	1 1/2+ ☐
Potetgull (1 pose 100g = 7 dl)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7+ ☐
Annen snacks (skruer, crisp, saltstenger, lettsnacks o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7+ ☐
Peanøtter, andre nøtter (1 pose 100g = 4 never)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(neve)	1 ☐	2 ☐	3 ☐	4+ ☐

15. KOSTTILSKUDD (bs = barneskje, ts = teskje)

	Hele året	Bare vinter-halvåret	Gang pr. uke: 0	<1	1	2-3	4-5	6-7		Mengde pr. gang
Tran	☐	☐	☐	☐	☐	☐	☐	☐		1 ts ☐ 1 bs ☐ 1 ss ☐
Trankapsler	☐	☐	☐	☐	☐	☐	☐	☐	kapsler	1 ☐ 2+ ☐
Fiskeoljekapsler	☐	☐	☐	☐	☐	☐	☐	☐	kapsler	1-2 ☐ 3-4 ☐ 5-6 ☐ 7+ ☐
Multipreparater										
Sanasol	☐	☐	☐	☐	☐	☐	☐	☐	bs	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Biovit	☐	☐	☐	☐	☐	☐	☐	☐	bs	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Vitaplex	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Kostpluss	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Vitamineral	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Annet	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Hvis annet, hvilket?										
Jernpreparater										
Ferro C	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Hemofer	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Duroferon Duretter	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Annet	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Hvis annet, hvilket?										
B-vitaminer	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
C-vitamin	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
D-vitamin	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
E-vitamin	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Folat (folsyre)	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Kalktabletter	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Fluortabletter	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Annet	☐	☐	☐	☐	☐	☐	☐	☐	tablett	1 ☐ 2 ☐ 3 ☐ 4+ ☐
Hvis annet, hvilket?										

16. NÅR SPISER DU PÅ HVERDAGER?

HOVEDMÅLTIDER som frokost, formiddagsmat, middag, kvelds.

Omtrent klokken

6	8	10	12	14	16	18	20	22	24	2	4
☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐

MELLOMMÅLTIDER som kaffe, frukt, godteri, snacks m.v.

Omtrent klokken

6	8	10	12	14	16	18	20	22	24	2	4
☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐

17. MENER DU SVARENE I SPØRRESKJEMAET GIR ET BRUKBART BILDE AV KOSTHOLDET DITT?

Ja ☐ Nei ☐

Er det matvarer/produkter du regelmessig bruker, og som ikke er nevnt i skjemaet?

.....

.....

18. ER DU FORNØYD MED KROPPSVEKTEN DIN SLIK DEN ER NÅ?

- ☐ Ja
- ☐ Nei, jeg ønsker å slanke meg
- ☐ Nei, jeg ønsker å legge på meg

19. KJØNN

Mann ☐ Kvinne ☐

Vennligst se etter at du har svart på alle spørsmål.

Takk for innsatsen!