Influence of dietary and cardiovascular risk factors on components of one-carbon metabolism

A study on predictors of plasma homocysteine, choline and betaine

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Dissertation for the degree philosophiae doctor (PhD)

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Contents

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SUMMARY

The basic theme of the dissertation is components of methionine and lipid metabolism including homocysteine, choline and betaine, and their relation to nutrition and cardiovascular disease (CVD) risk factors. Established predictors of plasma total homocysteine (tHcy) include several CVD risk factors and B-vitamins, while information is scarce regarding predictors of plasma choline and betaine. The aim of this study was to examine the role of diet, lifestyle and other CVD risk factors in prediction of plasma choline and betaine in a large population-based study. The study population included 7074 men and women aged 47-49 and 71-74 years who participated in the Hordaland Health Study conducted in 1997-1999.

Plasma tHcy levels were inversely related to the intake of fruit and vegetables, bread, fish, non-processed meat and eggs and positively related to products rich in sugar and fat. High intakes of saturated, monounsaturated and polyunsaturated fat were related to higher tHcy, with the exception of very long chain n-3 fatty acids [VLC n-3 FA] which was inversely related to plasma tHcy. The latter association may be explained by simultaneous consumption of B-vitamins and by increased plasma betaine concentration.

Plasma choline was positively associated with the intake of eggs, low fat-milk and beer and inversely related to the intake of legumes, sugar products and coffee. Plasma choline was determined by only one nutrient, cholesterol.

Plasma betaine was positively associated with the consumption of bread and tea, and inversely associated with sugar- and fat-rich products, non-processed meat and alcohol. Saturated and monounsaturated fat was inversely and VLC n-3 FA positively related to plasma betaine. The latter association was confounded by dietary folate, vitamin supplement

use and physical activity, perhaps reflecting a healthier lifestyle among individuals consuming VLC n-3 FA. In addition, betaine was positively related to total energy intake. A dietary pattern high in fat and sugar was associated with lower betaine concentration.

Plasma choline and betaine were associated with CVD risk factors including major components of the metabolic syndrome. Choline was positively associated with serum triglycerides (TGs), glucose and body fat, and inversely related to HDL cholesterol and smoking. Betaine was inversely associated with serum non-HDL cholesterol, TGs, body fat and blood pressure, and positively associated with HDL cholesterol and physical activity. Thus, an unfavourable cardiovascular risk factor profile was associated with high choline and low betaine concentrations. Divergent associations with choline and betaine may be explained by a disruption of the mitochondrial choline dehydrogenase pathway under conditions of mitochondrial dysfunction in the metabolic syndrome.

Diet influences the concentrations of both plasma tHcy and betaine. A diet low in B-vitamins and with a high content of saturated fat and sugar may decrease betaine and increase tHcy levels. On the other hand, a diet rich in B-vitamins and VLC n-3 FAs may increase betaine, leading to decreased tHcy concentrations and hence, decrease the risk for CVD.

LIST OF PAPERS

PAPER I Svetlana V. Konstantinova, Stein Emil Vollset, Paula Berstad, Per M. Ueland, Christian A. Drevon, Helga Refsum and Grethe S. Tell. 'Dietary predictors of plasma total homocysteine concentration in the Hordaland Homocysteine Study', British Journal of Nutrition, 2007; 98: 201-210.

PAPER II Paula Berstad, Svetlana V. Konstantinova, Helga Refsum, Eha Nurk, Stein Emil Vollset, Grethe S.Tell, Per M. Ueland, Christian A. Drevon and Giske Ursin. 'Dietary fat and plasma total homocysteine concentration in 2 adult age groups: The Hordaland Homocysteine Study', American Journal of Clinical Nutrition, 2007; 85: 1598-1605.

PAPER III Svetlana V. Konstantinova, Grethe S. Tell, Stein Emil Vollset, Arve Ulvik, Christian A. Drevon and Per M. Ueland 'Dietary patterns, food groups and nutrients as predictors of plasma choline and betaine in middle age and elderly men and women'. Under second review in the American Journal of Clinical Nutrition

PAPER IV Svetlana V. Konstantinova, Grethe S. Tell, Stein Emil Vollset, Ottar Nygård, Øyvind Bleie and Per Magne Ueland. 'Divergent associations of plasma choline and betaine with components of the metabolic syndrome in middle age and elderly men and women', The Journal of Nutrition, 2008; 138: 914-920.

ABBREVIATIONS

BD	Betaine aldehyde dehydrogenase
B_2	Riboflavin
B_6	Vitamin B_6
B_{12}	Cobalamin
Bet	Betaine
BHMT	Betaine homocysteine methyltransferase
BMI	Body mass index
CD	Choline dehydrogenase
CDP-Cho	Cytidine diphosphocholine
Cho	Choline
CI	Confidence interval
CH_2THF	Methylenetetrahydrofolate
CH_3THF	Methyltetrahydrofolate
CT	CTP: phosphocholine cytidylyltransferase
CVD	Cardiovascular disease
Cys	Cysteine
Cysta	Cystathionine
DMG	Dimethylglycine
HDL	High density lipoprotein
HHS	The Hordaland Homocysteine Study
HUSK	The Hordaland Health Study
LDL	Low density lipoprotein
Met	Methionine
MS	Methionine synthase

MTHFR	Methyltetrahydrofolate reductase
MUFA	Mono-unsaturated fatty acids
PE	Phosphatidylethanolamine
PEMT	Phosphatidylethanolamine methyltransferase
PUFA	Poly-unsaturated fatty acids
PC	Phosphatidylcholine
P-Cho	Phosphocholine
S-AdoMet	S-adenosylmethionine
S-AdoHcy	S-adenosylhomocysteine
SD	Standard deviation
SFA	Saturated fatty acids
TG	Triglycerides
THF	Tetrahydrofolate
tHcy	Total plasma homocysteine
VLC n-3 FA	Very long chain n-3 fatty acids
VLDL	Very low density lipoprotein

DEFINITIONS

Apoprotein	Protein component of the lipoprotein shell, which increases the water solubility of the lipoprotein.
Blood lipoprotein	Water-soluble macromolecules. Each lipoprotein particle is composed of a core of hydrophobic lipids such as cholesterol esters and triacylglycerols surrounded by a shell of polar lipids (the phospholipids).
Cardiovascular disease	CVD is caused by disorders of the heart and blood vessels, and includes coronary heart disease, cerebrovascular disease, increased blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure.
Diabetes mellitus	A disorder of carbohydrate metabolism in which sugars in the body are not oxidized to produce energy due to lack of the pancreatic hormone insulin. The accumulation of sugar leads to its appearance in the blood (hyperglycaemia), then in the urine.
Diabetes mellitus, type 2	Noninsulin dependent DM, which usually occurs after the age of 40, the pancreas retains some ability to produce insulin but this is inadequate for the body's needs; alternatively, the body becomes resistant to the effects of insulin.
Essential nutrient	No biochemical pathways exist for the synthesis of this nutrient in humans, and hence this nutrient is only obtained from diet.
Glucose intolerance	Abnormal glucose tolerance
Insulin	Protein hormone, produced in the pancreas by the beta cells of the islets of Langerhans, which is important for regulating the amount of sugar (glucose) in the blood.
Insulin resistance	A state (of a cell, tissue, system, or body) in which greater-than-normal amounts of insulin are required to elicit a quantitatively normal response.
Methionine metabolism	The sum of the biochemical changes undergone by methionine in the body.
Metabolic syndrome	Constellation of lipid and non-lipid risk factor of metabolic origin: obesity, elevated blood pressure, impaired fasting glucose, and high TG and low HDL cholesterol concentrations.
Monounsaturated fatty acids	Contain one double bond between the carbons in the chain.

Polyunsaturated fatty acids	Fatty acids with two or more double bonds between the carbons in the chain.
Saturated fatty acids	Have single bonds between the carbons in the chain.
Unsaturated fatty acids	Contain one or more double bonds between carbons in the chain.
Very long chain n-3 fatty acids	Polyunsaturated fatty acids with 18-28 carbon atoms in the chain. The first double bond occurs between the 3rd and the 4th carbons.
Very low density lipoprotein	Is produced in the liver, mainly from dietary carbohydrate, and secreted into the blood. VLDL transports endogenously synthesized lipids to the tissues. VLDL particles are denser, as they contain a lower percentage of triglyceride than do the chylomicrons.

1 INTRODUCTION

Modifiable risk factors for cardiovascular disease

CVD is the number one cause of death in individuals over 60 years globally and this is projected to continue (1). An estimated 17.5 million people died from CVD in 2005, representing 30% of all global deaths. Of these deaths, 7.6 million were due to heart attacks and 5.7million were due to stroke. Heart attacks and strokes are mainly caused by a blockage that prevents blood flow to the heart or to the brain, mostly caused by build-up of fatty deposits in the blood vessel walls. The most important modifiable risk factors for heart disease and stroke are cigarette smoking, unhealthy diet and physical inactivity, responsible for about 80% of CVD. The effect of unhealthy diet and physical inactivity may be raised blood pressure, increased blood glucose and lipid levels, as well as overweight and obesity. These are sometimes referred to as 'intermediate risk factors' for CVD.

The World Health Organization (WHO) has defined overweight and obesity in adults as body mass index (BMI) of 25.0-29.9 kg/m^2 and $\geq$ 30.0 kg/m^2 , respectively (1, 2). Overweight and obesity have reached epidemic proportions globally along with an adoption of a westernized lifestyle characterized by a combination of excessive food intake and inadequate physical activity (1, 3). The dramatic rise in the prevalence of obesity has been accompanied by increases of incidence and prevalence of type 2 diabetes (2). Findings from epidemiological studies have repeatedly confirmed a strong positive association between excess adiposity and risk of developing type 2 diabetes (1). Because obesity and diabetes are major causes of morbidity and mortality, reversing the obesity and diabetes epidemics is of utmost importance (1). The increasing prevalence of obesity and diabetes in most parts of the world has imposed an enormous burden on health care systems, and this is expected to escalate in the future.

Homocysteine, choline and betaine and the risk for disease

Homocysteine

Elevated plasma total homocysteine (tHcy) concentration has been related to several adverse conditions and diseases (4, 5) including adverse pregnancy outcomes (6, 7), cognitive dysfunction among the elderly (8, 9) and osteoporosis (10, 11). Plasma tHcy is also a risk factor for CVD in prospective studies (12-15), and several studies have found associations between tHcy and coronary disease, cerebrovascular complications and peripheral arterial disease (16-21). While supplementation with B-vitamins effectively lowers tHcy concentrations, interventional trials with B-vitamin supplementation have failed to reduce risk in patients with established CVD, but there seems to be a beneficial effect in stroke (22-28).

Choline

Previous studies have reported that choline supplementation reduces tHcy (29, 30), and increases blood triglycerides (31). Although a few prospective studies have showed that dietary choline intake is not associated with CVD risk reduction (32, 33), the relation between choline and CVD is not clear. Experimental studies have found that choline deficiency is associated with non-alcoholic fatty liver disease (34, 35), which is common in obesity, type 2 diabetes mellitus and the metabolic syndrome (36, 37).

Betaine

Dietary supplementation with betaine may substantially decrease tHcy concentration (38-40), especially after methionine loading (when folate supplementation does not lower tHcy) (40-42). The reduction of tHcy by betaine supplementation is accompanied by increased LDL cholesterol and total/HDL cholesterol ratio (31, 43), while no effect on body composition and weight was seen in one study (39). Longitudinal observational studies found no association

between dietary betaine intake and CVD risk (32, 33). Little is known about the role of plasma betaine in disease, although increased BMI, body fat and plasma ApoB have been related to lower plasma betaine in patients attending a lipid clinic (44).

Biological function of homocysteine, choline and betaine

Homocysteine

Homocysteine (Hcy) is a non-protein sulfhydryl-containing amino acid in human tissues and plasma, and its only dietary precursor is the essential amino acid methionine. Plasma tHcy is a marker of folate status. In addition, via endogenous synthesis of phosphatidylcholine, homocysteine is related to lipoprotein synthesis and lipid metabolism (45).

Choline

Choline, which is an epigenetic regulator of gene expression (46), has a variety of other biological functions. It is a precursor of lipoproteins, membrane phospholipids and the neurotransmitter, acetylcholine; is therefore important for lipid metabolism, the integrity of cell membranes and nerve function (47). Phosphatidylcholine (lecithin, [PC]) is the most abundant choline derivative in humans (48), present in most human cells and tissues (49). Thus, choline is needed for the growing body, for brain development and function, lifelong memory function, normal muscle function, and it may influence the risk for neural tube defects (50-52).

In addition, phosphatidylcholine is required for the synthesis and secretion of very low-density lipoproteins (VLDL) to blood. VLDL transport fatty acids (FAs) in the form of TGs from the liver to peripheral tissues. FAs are an important source of fuel for muscles and they are oxidized for energy in muscular mitochondria or stored in adipose tissue. After

supplementation with PC the level of plasma TGs may be increased (29). On the other hand choline deficiency may also lead to increased TGs in liver and fatty liver disease (35, 48, 53, 54). Recently, choline has been considered as an essential nutrient for humans (48, 49, 51, 52) and recommended dietary allowances have been published (50, 55).

Betaine

Betaine has two functions in humans. First, it is an organic osmolyte that accumulates in a variety of cells, including renal medullary cells, under condition of hypertonicity (56). Secondly, it serves as a methyl donor in the betaine-homocysteine methyltransferase (BHMT) reaction (57), which is responsible for the betaine-dependent remethylation of homocysteine to methionine. This explains the inverse relationship between plasma tHcy and plasma betaine (41, 58), and why plasma concentration of tHcy decreases after betaine supplementation (42). Compared to folate, betaine supplementation may substantially reduce homocysteine after a methionine load (41) and this effect is stronger in individuals with the MTHFR TT genotype (58).

One-carbon metabolism

Figure 1 provides an overview of the link between methionine (Met) and lipoprotein metabolism, with emphasis on components related to tHcy, betaine and choline. Choline and betaine are obtained from food or synthesized de novo. PC is formed endogenously from phosphatidylethanolamine (PE) by a S-adenosylmethionine(S-AdoMet)-dependent methylation reaction catalyzed by phosphatidylethanolamine *N*-methyltransferase (PEMT). This is a significant source of choline relative to dietary intake. Hcy is formed during the S-adenosylhomocysteine(S-AdoMet)-dependent methylation of PE to PC. Three molecules of S-AdoMet are required for the synthesis of one molecule of PC, and three molecules of S-

AdoHcy are formed as a result of the reaction. Thus, Hcy is an intermediate amino acid in the metabolism of the amino acid Met (21) and a only source of Hcy in mammals is S-AdoHcy (22). The concentration of tHcy in plasma can be lowered by enhanced remethylation of Hcy into Met or via degradation through the transsulphuration pathway. Folate (5-methyltetrahydrofolate) or betaine donate a methyl group, while vitamins B_{12}, B_6 and riboflavin act as cofactors for enzymes involved in the Hcy metabolism (21, 57, 59). Thus, Hcy exists at a point of convergence of several B-vitamins, which explains their effects as tHcy lowering agents. Betaine is formed in kidney and liver by choline oxidation catalyzed by the mitochondrial enzyme, choline dehydrogenase (47, 60, 61). Both PC and betaine syntheses are under control of hormones, including sex hormones (estrogen, androgen) and insulin.

Predictors of plasma homocysteine, choline and betaine

Elevated tHcy is observed under a variety of conditions associated with lifestyle and CVD risk factors, e.g. deficiency of B-vitamins (21, 62), cigarette smoking (63, 64), high coffee consumption (62, 65) and impaired renal function (66). The role of choline and betaine in disease has only more recently been focused upon, and less is known about predictors of plasma choline and betaine levels.

Diet

Homocysteine. Several studies report an association between intake of different nutrients and plasma tHcy concentration (21, 62, 67). Two large studies; the Framingham Offspring study (n = 1960) and the Dutch population study (n = 2435) have found inverse associations between plasma tHcy and intake of folate, vitamin B12, B6, and riboflavin (68, 69). Also, mandatory food fortification with folic acid has increased plasma folate and decreased tHcy

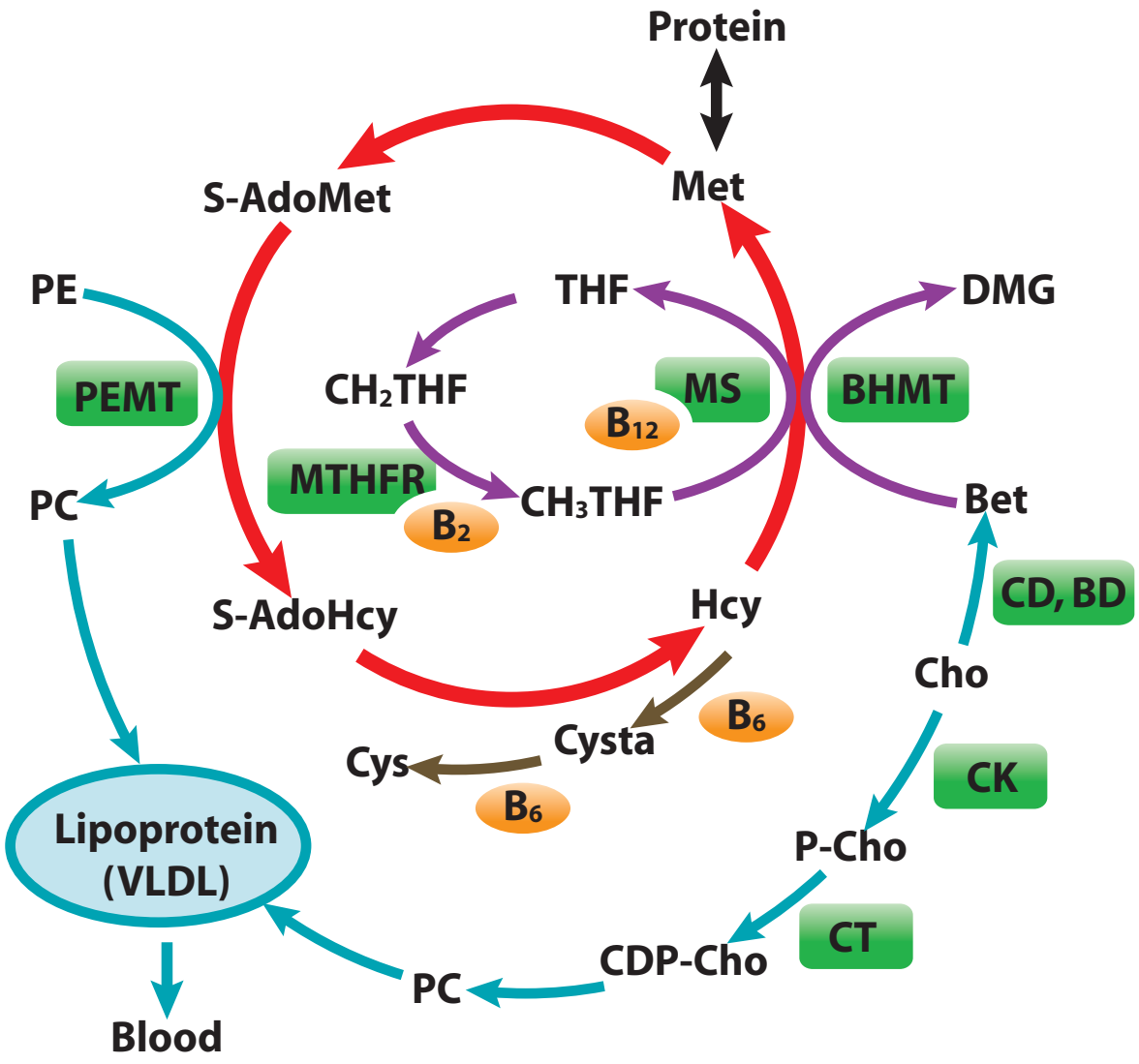

FIGURE 1. *The methionine and lipid metabolism.*

Phosphatidylcholine (PC) is formed from choline (Cho), which is obtained through the diet, or synthesized de-novo from phosphatidylethanolamine (PE). The latter reaction is catalyzed by the S-adenosylmethionine-dependent enzyme, phosphatidylethanolamine N-methyltransferase (PEMT). PC can also be synthesized via two-step the so-called Kennedy pathway (K-P), where CTP:phosphocholine cytidylyltransferase (CT) is the key regulatory enzyme, and phosphor-choline (P-Cho) and cytidine diphosphocholine (CDP-Cho) are formed prior to PC. PC is further used for lipoprotein formation (very low density lipid [VLDL]). Betaine (Bet) is generated from choline by the sequential action of mitochondrial choline dehydrogenase (CD) and betaine aldehyde dehydrogenase (BD). Betaine serves as a methyldonor in the betaine-homocysteine methyltransferase (BHMT) reaction, which catalyzes the remethylation of homocysteine (Hcy) to methionine (Met); dimethylglycine (DMG) is the other product of this reaction. Hcy remethylation is also catalyzed by the ubiquitous vitamin B12-dependent enzyme, methionine synthase (MS), which requires 5-methyltetrahydrofolate (mTHF) as methyl donor.

concentrations in the general US population (70, 71). Although less studied, dietary choline and betaine have been inversely related to plasma tHcy (62, 72), while there are contradictory reports on the relation of protein and methionine to plasma tHcy concentration (62).

The few studies that have examined the associations with total fat, cholesterol, saturated fat, mono- and poly-unsaturated fat (73), and omega-3 fatty acids (74) did not find statistically significant associations with tHcy concentrations. Results from an Irish study in which dietary patterns were related to plasma tHcy concentration, suggested a positive association between SFA intake and tHcy (75). The association between plasma tHcy and dietary fish and VLC n-3 FAs has been studied with inconsistent findings (74, 76-79). It has been hypothesized that high intake of n-3 fatty acids may reduce tHcy but only in combination with a high B-vitamin intake (12). There are even fewer studies of other dietary fat types. One study found that consumers of skim milk had lower plasma tHcy concentrations than non-consumers (80), suggesting an positive association between saturated fat intake and plasma tHcy.

Studies have shown that tHcy concentration is inversely related to the intake of food groups such as bread (67), cereal (73, 81), fruit (67, 82), vegetables (67, 74, 82), and to individual food items including cruciferous vegetables (81, 82), peppers (81), citrus fruits and juices (67, 82), cold breakfast cereals (73, 81, 82), milk (73, 81), yoghurt (81), and liver (67). Positive associations have been found with caffeine-containing drinks such as coffee (62, 69), Coca Cola (69) and tea (62, 69), while findings on alcohol are conflicting (67, 69).

Studies that have investigated associations between plasma tHcy levels and dietary patterns (83-87) have found that a Western type dietary pattern, characterized by high consumption of products high in fat and sugar, is related to higher tHcy levels (84, 86, 87), while a diet high

in folate and B-vitamins is associated with lower tHcy concentrations (83-85).

Choline and betaine. Choline and betaine may be obtained from the diet. Choline, the major source of methyl groups in the diet, is found in eggs, beef, pork, liver, soybean and wheat germ, whereas foods with the highest content of betaine are wheat bran, wheat germ and spinach (47, 88).

Several studies have found inverse associations between dietary choline and betaine and plasma tHcy level (72), and supplementation with choline and betaine has been shown to reduce tHcy concentration (29, 38). However, the mechanism through which dietary choline and betaine may affect plasma tHcy is not known. The relation between dietary choline and betaine and plasma levels of respectively choline and betaine, has not been investigated in epidemiological studies. Also, the relations of plasma choline and betaine to dietary patterns or intake of individual foods and beverages have not been investigated.

CVD risk factors

Homocysteine. Plasma tHcy shows a positive relation to a variety of CVD risk factors (5, 89). tHcy concentrations increase by age, and are higher in men than women (4). It is increased under condition of impaired renal function (21, 90) and is positively related to plasma creatinine (66). Recent studies have demonstrated that plasma tHcy increases with the number of components of the metabolic syndrome (91, 92), which refers to the cluster of risk factors related to central obesity, including elevated blood glucose, dyslipidemia and hypertension (93).

Choline and betaine. Little is known about the relation of CVD risk factors to choline and betaine. In postmenopausal women, dietary intake of betaine and choline was found not to be associated with CVD risk (33), while dietary intake of choline and betaine in healthy adults was associated with decreased concentrations of inflammatory markers (94). In a study among patients attending a lipid clinic, betaine was inversely associated with body fat and Apo B (44).

Rationale for the present study

Elevated tHcy is associated with an increased risk for hospitalisations due to CVD (95) and increased risk for death (4, 96). Randomized clinical trials have not found a risk reduction by tHcy lowering therapy with high-doses of B-vitamins in patients with established CVD (22-24). A relation between tHcy and dietary habits in community-dwelling adults and elderly have previously been investigated only in a few studies (97). We therefore wanted to study the effect of diet on plasma tHcy level in a large population-based study of more than 5800 men and women aged 47-49 and 71-74 y, in Norway where food is not fortified with B-vitamins. To understand the associations between plasma tHcy concentration and foods with high and low B-vitamin content, we included major food groups and individual food items in our study. Moreover, we examined the association between dietary intake of folate, vitamin B_{12}, B_6, riboflavin and other nutrients and plasma concentration of tHcy. We included nutrients other than B-vitamins to examine whether these might have an additional effect on tHcy concentration. This latter was possible because our dietary data allowed us to quantitatively assess the possible confounding effect of B-vitamins.

The association between fat intake and plasma tHcy has not been previously studied in a large population-based study. In the present study we addressed the question whether dietary fat

and especially VLC n-3 FA, mainly present in fish and marine oils, are associated with tHcy concentrations. Because there is experimental evidence that PC synthesis via the PEMT pathway can be increased by dietary fat (98), we hypothesized that the intake of different fat types may have different effects on PC synthesis and further on plasma tHcy concentration.

The role of plasma choline and betaine in cardiovascular disease is not well known, nor is the association of CVD risk factors, including diet, with plasma concentrations of these two metabolites known. The Hordaland Health Study afforded the examination of plasma choline and betaine in relation to individual foods, beverages, nutrients, and dietary patterns, as well as to CVD risk factors including several components of the metabolic syndrome.

2 AIMS OF THE STUDY

This dissertation addresses the epidemiology of nutrition and other CVD risk factors in relation to components of one-carbon metabolism, i.e. homocysteine, choline and betaine, in middle age (47-49 y) and elderly (71-74 y) men and women in a large population-based study in Norway.

The specific aims were to:

1. Assess the relationship between nutrients and food intake on plasma concentration of tHcy
2. Evaluate the association of dietary fat and especially VLC n-3 FAs with plasma tHcy concentration
3. Describe the population distribution of plasma choline and betaine concentrations
4. Assess the relations between individual dietary factors (foods, beverages and nutrients) and major dietary patterns on plasma choline and betaine
5. Investigate the association of plasma choline and betaine with CVD risk factors, including components of the metabolic syndrome

3 STUDY POPULATION AND METHODS

Study population

The Hordaland Health Study (HUSK) was conducted from 1997 to 1999 as a collaboration between the National Health Screening Service (now the Norwegian Institute of Public Health), the University of Bergen, the University of Oslo and local health services in the Bergen area. Among those invited were subjects who had previously participated in the Hordaland Homocysteine Study (HHS I) during 1992-93, and who were born 1925-27 and 1950-51. This second round of the Hordaland Homocysteine Study is referred to as HHS II, and participants here comprise the study population in the articles included here. Of the total sample of 9187 men and women who were invited to participate in HUSK, 7074 (77%) agreed to participate. The participants underwent a brief health examination and donated a nonfasting blood sample. Information on diet and lifestyle was collected via self-administered questionnaires. In total 6145 subjects (87%) completed a food frequency questionnaire (FFQ); among these, plasma tHcy was measured in 6118 and plasma choline and betaine in 6112 (**Table 1**).

TABLE 1.

Description of exclusion criteria of participants included in the four articles comprising this dissertation

	Article/Study			
	1	2	3	4
Participants in HUSK	7074	7074	7074	7074
Plasma tHcy measured	7053	7049	7045	7045
Plasma choline and betaine measured			7045	7045
Completed nutritional form	6145	6145	6145	6145
Number of participants excluded:				
Missing information of vitamin use	5	5		
Missing information of plasma tHcy	22	22		
Missing information of plasma choline and betaine			29	29
Missing information on dietary fatty acids			4	
Sample without missing information	6118	6118	6112	7045
Exclusion for energy intake*	301	201	300	
Remaining sample, n	5812	5917	5812	7045
%	82.2	83.6	82.2	99.6

* in study 1 and 3: below 2.5 percentile (2124 kJ for women 71-74 y and 3899 kJ for women 47-49 y; 3856 kJ for men 71-74 y and 5572 kJ for men 47-49 y) and above the 97.5 percentile (11098 kJ for women 71-74 y and 12970 kJ for women 47-49 y; 14023 kJ for men 71-74 y and 17590 kJ for men 47-49 y); in study 2 < 3000 kJ for women; < 3300 kJ for men and >15000 kJ for women; > 17500 kJ for men.

Study 1

We excluded participants with missing values for plasma tHcy (n = 22), dietary data (n = 933) and those with reported energy intake below the 2.5 percentile and above the 97.5 percentile. By these latter criteria, 141 men and 187 women were excluded, yielding a final number of 5812 participants (82% of those attending the health examination). Participants with reported seasonal or regular intake of at least one dose of a multi- and/or individual vitamin supplement (excluding fish oil and omega 3 fatty acids) per day were assigned to the vitamin supplement user group (n = 1234). Thus, the group of non-users of vitamins consists of 4578 individuals (65% of participating subjects).

Study 2

After excluding individuals with missing plasma tHcy values (n = 25) and dietary data (n = 933), participant with a very low and very high estimated daily energy intake, which left 5917 individuals (83.6% of those attending the health examination).

Study 3

In addition to excluding participants with missing values for plasma choline and betaine (n = 29) and nutritional data (n = 933) we excluded participants with reported energy intake below the 2.5 percentile and above the 97.5 percentile. By these latter criteria, 131 men and 169 women were excluded from the analyses, yielding a final number of 5812 (82% of participants).

Study 4

We excluded 29 persons with missing value for choline and betaine concentrations, thus the final number of participants was 7045 (99.6% of 7074).

Health survey

The health examination included measurements of height and weight, as well as waist and hip circumferences, following standard protocols used by the National Health Screening Service. Blood pressure was measured after 10 min seated resting using Dinamap 845 XT equipment (Criticon, Tampa, Fla), and the mean values of three measurements were used for analyses. Body composition including percent body fat was measured by dual energy x-ray densitometry (EXPERT-XL; Lunar Company Inc, Madison, Wis) (99). Level of physical activity was defined as a combination of intensity and frequency: no, low, moderate and high activity. Smoking status was defined as 'current smokers' and 'non-smokers (including former smokers)' in study 2, 3 and 4, while we used three groups 'current smokers', 'former smoker' and 'non-smokers' in study 1. At the time of the blood draw, time since last meal was recorded (from 0 to 11 hours).

Assessment of diet

Dietary data were collected by using a 169-item FFQ, a slightly modified version of a FFQ previously described in detail (100). This FFQ aimed to capture the habitual diet during the past year and included frequency alternatives (from once a month to several times per day), the number of portion eaten and portion sizes (e.g. slices, glasses, cups, pieces, spoons).

The information from the FFQ is presented as individual food items, food groups (consisting of several individual food items) and nutrients. Individual food items correspond to the items listed on the questionnaire, whereas 'food groups' include related food items. The FFQ also included nine questions about vitamin supplements on the market at the time of the study.

Subjects using at least one dose of vitamin supplement per day seasonally or regularly during the past year were classified as vitamin supplement users.

Daily nutrient intake was computed from a database and software system developed at the Department of Nutrition, University of Oslo (Kostberegnings-SYSTEM, version 3.2). The nutrient database was mainly based on the official Norwegian food composition table with an update on folate content from 2001 (101). B-vitamin intake in supplements was calculated from information on the contents of vitamin supplements for sale during 1997-99. We assessed daily consumption of the main food groups and items contributing to the intake of major macro- and micro-nutrients, including vitamins (folate, vitamins B_{12} and B_6, riboflavin, thiamine, α-tocopherol, β-carotene, retinol equivalents, vitamins D and C), and different types of fat (saturated fatty acids [SFA], monounsaturated fatty acids [MUFA], and the polyunsaturated fatty acids [PUFA], n-6 PUFA, n-3 PUFA and VLC n-3 PUFA). For the dietary pattern anlysis we assigned each food item into one of 41 non-overlapping food groups (**Table 2).**

TABLE 2.

Definition of food groups from the food frequency questionnaire items

Food groups	FFQ items contributing to food group
Refined bread	
Medium-fiber bread	
High-fiber bread	
Breakfast cereals	Oat, rice flakes, mixture of cereals, porridge, MUSLI
Flour, rice, pasta	Wheat, rye, oat, wheat germ, rice, pasta
Pizza	With and without meat
Cakes	Cakes, cookies, wafflers, Danish
Potatoes	Boiled, baked, fried, pommes frites
Carrots	
Cabbage, kohlrabi	
Cauliflower, broccoli	Brussel sprouts
Onions, spinach	Garlic, parsley
Cucumber, tomato, pepper, lettuce	
Vegetables, raw other	
Vegetables mixed, frozen and preserved	
Mushrooms	
Beans, peas, dried	
Fruit	Fruits and berries, fresh, dried or canned with sugar
Juice	From fruits and berries
Red meat, whole	Beef, pork, lamb, game meat, minced meat
Processed meat	Sausages, meat balls, meat puddings, other meat

	products
Liver	Liver paté
Chicken	
Eggs	
Lean fish	Cod, saithe, haddock, catfish, pollack
Fatty fish	Salmon, trout, herring, mackerel, halibut
Processed fish	Fish balls, fish pudding, other fish products
Shellfish	Shrimp, crab, mussels, roe, fish liver
Milk	Whole and reduced fat, sour milk (kefir)
Yoghurt	Whole and reduced fat and sugar, yoghurt with fruits
Cheese	White cheese, cottage cheese, blue cheese, goats cheese, other cheese, brown (whey) cheese
Cream, ice cream	Sour cream, whipping cream, milk desserts, pudding
Margarine, butter	High, reduced and low fat, soya
Oils	Vegetable, cooking, olive, maize, soy, sunflower,
Mayonnaise	High and low fat dressing
Sugar, sweets	Diet sugar, chocolate, honey, candy, other sugar products
Snacks	Popcorn, potato chips nachos, nuts, other snacks
Coffee	
Tea	
Soft drinks with sugar	Soda with sugar and diet soda
Alcoholic beverages	Beer, wine, liquor

Analytic procedures

Non-fasting blood samples used for the preparation of plasma were collected into evacuated tubes containing EDTA, placed in a refrigerator (4-5°C) within 15-30 minutes, and centrifuged, usually within 1 hour (maximum within 3 hours). EDTA plasma was stored at -80° C. For study 1 and 2 plasma tHcy was determined by automated HPLC with fluorescence detection. Intra-assay coefficient of variation was 3% (102). For study 3 and 4 plasma choline, betaine, tHcy and creatinine were determined by a method based on normal-phase chromatography-tandem mass spectrometry (LC-MS/MS) (103). For these two studies we used the new measurement values for tHcy and creatinine because they were included in the new platform and were measured at the same time as choline and betaine. The concentration of plasma folate was measured by a *Lactobacillus casei* microbiological assay (104) and plasma vitamin B12 concentration by a *Lactobacillus leichmannii* microbiological assay (105).

Blood samples used for the preparation of serum were collected into evacuated tubes containing sodium sulphite titration gel and centrifuged within two hours. The serum tubes were transported to the department of Clinical Chemistry, Ullevål University hospital, Oslo, where determination of glucose, total cholesterol, HDL cholesterol and triglycerides were performed, using enzymatic methods with reagents from Boehringer Mannheim, Federal republic of Germany as adapted to a Hitachi 911 analyzer. HDL cholesterol was measured by a direct, enzymatic inhibition method. Serum lipids were determined within seven days after collection of the sample. The concentration of serum creatinine was measured with standard alkaline picrate colorimetric assay.

Statistical methods

Several statistical methods were used for data analyses in the study. Descriptive methods were used to evaluate differences between population groups: men and women (study 1, 2, 3, 4), older and younger individuals (study 1, 2, 3, 4), vitamin supplements users and non-users (study 1). Spearman partial correlations were used to assess the relationship between dietary and plasma B-vitamins with foods and nutrients (study 1). The same method was used to evaluate the association between plasma choline and betaine (study 4). Multiple linear regression analyses were used to assess the effect of independent variables on the outcome variable (study 1, 2, 3, 4) and to evaluate possible interaction effects (study 1, 2). In addition, linear and logistic regression (study 1, 2) was used for comparing differences between study groups. The Gaussian generalized additive regression models (GAM) were used to assess dose-response relations between study and outcome variables (study 2). To define major dietary patterns principal component analysis (PCA) was used prior to factor analysis (study 3). PCA allows reduction of the number of study variables to principal components (factors) (106). Three factors were extracted to produse three dietary patterns which were used in futher analyses.

In general several adjustment models were used to control for possible confounding. The first model was adjusted for age and gender (study 4) and energy intake (study 1, 2, 3). Second and third models included additional adjustments for smoking, coffee consumption, physical activity, BMI, intake of fruits and vegetables, dietary or/and plasma B-vitamins, time since last meal and plasma/serum creatinine.

All statistical analyses in study 1, 3 and 4 were performed using SAS for Windows v 9.1 (SAS Institute Inc., Cary, NC, USA). In study 2, we used SPSS for WINDOWS software,

release 12.0.1; SPSS Inc., Chicago, IL). Gaussian generalized additive regression models (30) was implemented in S-PLUS for WINDOWS (version 6.2; Insightful Corporation, Seattle, WA). In addition, we used augmented convex hull plots as implemented in the programme "R" for graphical presentation of choline and betaine distributions in study 4 (107).

We initially conducted all analyses stratified by the four age and sex groups. Differences in the consumption of food groups and nutrients between age groups and sex and between users and non-users of vitamin supplements were assessed by linear regression for continuous variables and logistic regression for categorical variables. In study 2 potential differences in tHcy, BMI and dietary factors between gender and age groups were tested by univariate analysis of variance and logistic regression. In study 3, differences in plasma choline, betaine, creatinine, BMI, foods and nutrients were assessed by Wilcoxon rank sum tests. In study 4, we used independent sample t-tests for assessing differences in plasma choline and betaine between sexes and age groups.

In multiple linear regression analyses, the continuous predictor and covariate variables were categorized in quartiles, except time since last meal, and with the exception of some variables (foods and beverages) for which a high proportion of the subjects (more than 50% of participants) reported 'no use'. For these variables three categories were used: 'non-users' and two equally large groups of users. Thus, the regression coefficients estimated the mean (95% CI) differences in plasma tHcy, choline and betaine concentrations per increasing quartile or contrasting group of the predictor variables (foods, beverages, nutrients, dietary pattern scores, lifestyle factors, body composition, blood pressure, serum lipids and glucose, plasma folate, vitamin B_{12}, tHcy and creatinine). All *P* values were 2-sided and values < 0.05 were considered significant. Categories of predictors were defined separately for each of the

four age-sex groups, two age groups and users and non-users of vitamin supplements. Results were combined for the two age groups and for men and women in study 1 and 3, because the observed trends were similar in all the four age-sex groups.

In the first study, we found an interaction effect of vitamin user group on the associations with several food groups and nutrients. Thus, due to these results and the fact that B-vitamin supplementation may substantially reduce plasma tHcy concentration and therefore mask the effects of dietary components, we restricted the study sample to non-users of vitamin supplements in further in-depth analyses.

In the second study, for the association between tHcy and dietary factors in the two age groups, we used multiple linear regression analysis to estimate the least squares mean of tHcy concentrations by categories of dietary factors. tHcy values were $\log_{10}$ transformed, and back transformed means and 95% confidence intervals are presented. Because we observed several significant interactions by age group, but not by sex, results are presented for the whole group, as well as for the two age groups.

For testing of the interaction between B-vitamins and n-3 FA intake, we constructed a summary score for total B-vitamin intake, which was calculated as the sum of quartile scores (1-4) for intake of folate and riboflavin and vitamins B_6 and B_{12}. Interaction between the quartiles of total B vitamin intake and the intake of these FAs was analyzed by multiple regression, which included a product term of the quartile of n-3 FA or VLC n-3 FA intake and the total B-vitamin intake score. We also evaluated the intakes of n-3 and VLC n-3 FAs as tHcy predictors in strata of B-vitamin intake by carrying out multiple regression analyses in the separate quartiles of B-vitamin intake.

Gaussian generalized additive regression models (108) were used to generate graphic representations of the dose–response relations between tHcy concentrations and the intake of different types of fat.

Three factors (dietary patterns) were extracted using PCA based on eigenvalues > 2, the scree plot and the natural interpretation of the factors after orthogonal (Varimax) rotation. The derived factors were labelled on the basis of knowledge of Norwegian dietary habits and nutritional science as “Western”, “Healthy” and “Traditional”. The factor score for each pattern was calculated by summing intakes of food groups weighted by their factor loadings, and each participant received a factor score for each identified pattern. We categorised participants by quartiles of dietary pattern scores. Mean differences in plasma choline and betaine per increasing quartiles of the three defined dietary pattern scores were calculated in linear regression analyses.

Approvals

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

4 SUMMARY OF RESULTS

Major dietary predictors of plasma tHcy

Plasma homocysteine in users and non-users of vitamin supplement.

The first study aimed to assess dietary predictors of plasma tHcy in a Norwegian population sample. It is important for this study question to note that food is not fortified with B-vitamins in Norway. Because vitamin use and lifestyle in general may have complementary effects on tHcy level, we first investigated differences between users and non-users of vitamin supplements and secondly, differences in associations between tHcy and dietary predictors in these two groups. Approximately 21% of participants reported the use of vitamin supplements (not including fish and fish liver oils). Compared to non-users, there was a higher proportion of women among users of vitamin supplements ($P < 0.0001$). Moreover, supplement users had a lower proportion of smokers, lower BMI and coffee consumption, and higher intakes of cereals, fruits, vegetables and fish compared to non-users. The intake of vitamins from the diet, including B-vitamins, was also higher in users compared to non-users of vitamin supplements. The former group also had lower concentrations of tHcy ($P < 0.0001$), whereas plasma folate and vitamin B12 were higher ($P < 0.0001$ and $P < 0.02$, respectively).

In addition, there were differences in the associations between food groups and nutrients with plasma tHcy between users and non-users of vitamin supplements. Therefore, users of vitamin supplements were excluded from further analyses. In non-users of vitamin supplements, plasma tHcy was more strongly predicted by plasma folate (r=- 0.37) and vitamin B12 (r=- 0.22), compared to dietary intake of folate (r=- 0.15), vitamin B12 (r=- 0.07), B6 (r=- 0.14) and riboflavin (r=- 0.12). Dietary folate was significantly positively correlated with the intake of vegetables, fruits and fiber, as well as with most other vitamins, including vitamin B6

(r=0.56), riboflavin (r=0.37), and vitamin B12 (r=0.16). On the other hand, intake of simple carbohydrates was inversely associated with dietary folate, B6 and riboflavin. In addition, consumption of fat was inversely related to the dietary folate, B6 and riboflavin, and to plasma folate (r=- 0.33).

Plasma homocysteine according food groups, food items and nutrient

Plasma tHcy was associated with several different nutrients and foods. Plasma tHcy level was lower not only with increased consumption of plant-origin food, vegetables, fruits and cereals, but also with intake of eggs, fish and milk, chicken and non-processed meats. The estimated mean difference in tHcy per increasing quartile of intake ranged from - 0.11 (95% CI - 0.21, - 0.01) μmol/l for milk to - 0.32 (- 0.42, - 0.22) μmol/l for vegetables. Furthermore, individual food items such as citrus fruits, orange juice, cruciferous vegetables, and spinach/green cabbage were related to lower plasma tHcy levels. Whole grain (medium- and high-fiber) bread had an additional inverse, but weaker effect on plasma tHcy level, while sugar products and cakes were positively related to tHcy concentration. Among nutrients, complex carbohydrates, protein and B vitamins (folate, vitamin B12, B6 and riboflavin) were associated with lower tHcy concentrations, while the intake of fat and sugar were associated with increased plasma tHcy.

Plasma homocysteine according to type of dietary fat

Plasma tHcy concentrations was inversely related to the consumption of lean fish, reduced fat milk, skim milk, vegetable oil and fish oil supplement. The magnitude of the differences in tHcy between the highest and the lowest quartile of intake of these foods ranged from 2.5% to 6.3 %. On the other hand, plasma tHcy concentration was positively associated with the consumption of total fat (*P* for trend from lowest to highest quartiles < 0.001), SFAs (*P* for

trend < 0.001), MUFAs (*P* for trend < 0.005) and PUFAs (*P* for trend = 0.03; significant only in the youngest age group). The difference in tHcy between the highest and the lowest quartiles of SFAs was 8.8%. In contrast, the intake of marine VLC n-3 FAs was inversely associated with tHcy level, the difference in tHcy between the highest and the lowest quartiles was – 5.0% (*P* for trend < 0.001).

The association between SFAs and plasma tHcy was independent of the effects of dietary folate and other B-vitamins (*P* < 0.001). However, there was an interaction between total intake of B vitamins (folate, B_6, B_{12} and riboflavin) and the total intake of n-3 FAs, as well as VLC n-3 FAs (*P*=0.01) with plasma tHcy concentrations. When stratified by quartiles of B-vitamin intake, the inverse trend between tHcy and VLC n-3 FAs intake was significant only in the highest quartile of B-vitamin intake. This was also observed when excluding users of fish-oil and B-vitamin supplements.

Major predictors of plasma choline and betaine

Plasma choline and betaine according age group and sex

Overall, plasma concentrations (mean±SD) of betaine were four times higher compared to plasma free choline 39.5±12.5 μmol/L versus 9.9±2.3 μmol/L, respectively. Plasma choline and betaine were positively correlated (r=0.37; *P* < 0.0001). Both choline and betaine were lower in women than in men, and in younger subjects compared to older (*P* < 0.0001).

Plasma choline and betaine according foods, beverages and nutrients

Levels of both choline and betaine were decreased with increasing time since the last meal. Plasma choline was positively associated with the consumption of individual food items such as eggs, skim milk and beer, and inversely related to legumes, coffee and sugar products.

Choline was not significantly associated with total energy intake, and the only nutrient associated with choline was cholesterol.

Plasma betaine was positively associated with intake of high-fiber bread and tea, and inversely related to non-processed meat, shellfish, whole milk, cream, butter, mayonnaise, sugar products, soft drinks and alcoholic beverages (except beer). It was also positively related to energy intake. With regard to nutrients, betaine was positively related to complex carbohydrates, fibers, folate and thiamine, and inversely associated with total, saturated and monounsaturated fat, as well as with alcohol.

Plasma choline and betaine according to dietary patterns

Three major dietary patterns were identified by factor analysis and denoted "Western", "Healthy" and "Traditional". Plasma choline was not significantly associated with any of these patterns, while betaine was inversely related to the "Western" dietary pattern, characterized by high loadings for pizza, pasta, meat, products high in fat and sugar including soft drinks, and alcoholic beverages ($P < 0.0001$). Controlling for potential confounders, such as smoking, physical activity and BMI did not materially change the association ($P = 0.0001$).

Plasma choline and betaine according components of the metabolic syndrome

Similarly to the findings of different associations between choline, betaine and diet, choline and betaine also showed divergent association with key components of the metabolic syndrome. Choline was positively associated with serum TGs, glucose, BMI, percent body fat and waist circumference ($P < 0.0001$ for all), and inversely related to HDL cholesterol ($P < 0.05$). On the other hand, betaine was inversely associated with serum non-HDL cholesterol, TGs, BMI, percent body fat, waist circumference, systolic and diastolic blood pressure ($P <$

0.0001 for all), and positively associated with HDL cholesterol ($P < 0.01$). Thus, an unfavourable cardiovascular risk factor profile was associated with high choline and low betaine concentrations. The associations were similar in the four sex-age groups, except for the positive relations of choline to BMI, percent body fat, and waist circumference, which were significant only among women, although a similar trend was seen also among men.

5 DISCUSSION

The basic aims of this dissertation were to examine plasma homocysteine, choline and betaine in relation to diet and other CVD risk factors. Dietary variables examined were food groups, food items and beverages, nutrients and dietary patterns. Moreover, because the distribution of plasma choline and betaine in the general population is not known, we also wished to examine this. The relation of plasma choline and betaine to smoking, physical activity, body mass index, percent body fat, waist circumference, blood pressure, serum lipids and glucose was also examined. Methodological strengths and limitations and to what extent bias and confounding may have influenced the results will be discussed. This is the largest observational study on diet as predictor of plasma tHcy, choline and betaine in a general population sample in a country in which food is not fortified with B-vitamins. It is also the first study of the relation of dietary patterns to plasma choline and betaine, and the largest study ever examining associations between major components of the metabolic syndrome and plasma choline and betaine.

Methodological considerations

In this section internal and external validity of the study will be discussed. The internal validity deals with the rigour of the theoretical, methodological and empirical bases underlying the research project (109, 110). In our study internal validity deals with the degree to which investigated relations and effects of exposure (diet, lifestyle and other CVD factors) on outcome measure (plasma tHcy, choline and betaine concentrations) is true for the study population. The possibility of confounding and bias will be discussed because of their potential effects on the findings. 'The external validity reflects the relevance and accessibility to user groups outside the study population' (109, 110), and refers to the degree to which results of the study may be extrapolated to the general population, also called generalizability.

Study design

Our studies were observational with a cross-sectional design. Exposure and outcome variables were measured approximately at the same time, thus inferences regarding cause and effect may not be drawn. In an ideal cross-sectional study, the study sample should reflect various health status and social-demographic groups in proportions distributed in the source population, location and time. The cross-sectional design with a reasonable high participation rate allows assessment of the prevalence of diseases and risk factors in a given population.

Selection bias

Selection bias may be introduced from procedures used to select individuals for the study and from factors influencing study participation. The relations between the exposure and outcome therefore may be different for the participating individuals and for individuals eligible for the study, but who do not participate.

All 9187 non-institutionalized persons from four communities in Hordaland County born 1925-27 and 1950-51 were invited to the second round of the Hordaland Homocysteine Study in 1997-99, as part of the Hordaland Health Study (HUSK). Thus, the study population was large and well defined. Analyses of a few socio-demographic characteristics for attendees and non-attendees in HUSK has been reported: http://www.uib.no/isf/husk/Vedlegg_dokumenter/FrafallsanalyseHUSK9799.pdf. However, these comparisons are for the HUSK study population as a whole, with no information specifically for the age groups included in this study.

Analysis of socio-demographic and other characteristics of HUSK participants who did and

did not fill out the FFQ showed that among those who did complete the questionnaire (the nutrition substudy group) there was a higher proportion of men in the oldest group, and a lower proportion men in the youngest group (**Tables 3 and 4**). Compared to those who did not complete the FFQ, a smaller proportion of the nutrition substudy group were smokers and physically inactive, and a smaller proportion had diabetes. In the youngest group, a larger proportion of the nutrition substudy participants had used medications the day before the survey. In the oldest group, the nutrition substudy participants consumed more alcohol and a smaller proportion had not completed high school. Thus, the HUSK participants who took part in the nutrition substudy had generally a healthier lifestyle compared to those who did not.

TABLE 3. Comparison between 71-74 year-old participants and non-participants in the nutritional substudy based on data from the Hordaland Health Study (HUSK 1997-99).

Selected variables	Participants N = 3017 %	Non-participants N = 330 %	p-value*
Men	44.7	39.1	0.05
BMI $\geq$ 30 kg/m^2	13.4	18.2	0.03
Systolic blood pressure $\geq$ 130 mmHg	80.8	78.5	0.30
Daily smoker	15.2	21.4	0.004
No and low physical activity**	37.5	47.9	0.0002
Alcohol $\geq$ 5 times/month	14.5	6.1	< 0.0001
Coffee $\geq$ 5 cups/d	8.1	7.3	0.59
Self defined health status as good and very good	14.4	9.2	0.19
Myocardial infarction	10.0	9.3	0.71
Diabetes mellitus	6.4	10.3	0.009
Angina pectoris	11.8	9.4	0.20
Stroke	4.3	6.6	0.06
Medicine use, yesterday	76.9	78.9	0.45
Did not complete high school	45.6	62.7	0.01

* evaluated by Chi-square test

** < 2 hours/week of non-intensive or < 1 hour/week of intensive physical activity.

TABLE 4. Comparison between 47-49 year-old participants and non-participants in the nutritional substudy based on data from the Hordaland Health Study (HUSK 1997-99).

Selected variables	Participants N = 3121 %	Non-participants N = 615 %	p-value*
Men	43.0	52.4	< 0.0001
BMI $\geq$ 30 kg/m^2	10.4	11.4	0.46
Systolic blood pressure $\geq$ 130 mmHg	40.6	44.2	0.10
Daily smoker	33.8	39.2	0.01
No and low physical activity**	24.8	31.4	0.0007
Alcohol $\geq$ 5 times/month	30.1	27.8	0.26
Coffee $\geq$ 5 cups/d	20.2	22.4	0.20
Self defined health status as good and very good	43.6	34.8	0.08
Myocardial infarction	0.6	0.8	0.42
Diabetes mellitus	0.8	2.0	0.008
Angina pectoris	0.9	1.0	0.85
Stroke	0.6	0.5	0.79
Medicine use, yesterday	53.0	46.0	0.002
Did not complete high school	6.1	7.0	0.80
Annual income $\leq$ 200.000 NOK	22.0	22.8	0.66

* evaluated by Chi-square test

** < 2 hours/week of non-intensive or < 1 hour/week of intensive physical activity.

Information bias

Incorrect information about exposure variables collected from participants may cause systematic error in the estimated relations. Information bias includes measurement errors and may cause misclassification. When misclassification is related to the exposure variable then associations between exposure and outcome may be strengthened or weakened by misclassification. On the other hand, when misclassification is not related to exposure or outcome, it has attenuating effect on the relation between exposure and outcome.

Food frequency questionnaire. The FFQ is a self-administered questionnaire which has a potential for misclassification of dietary intake (111, 112). This FFQ was developed to estimate the habitual diet during the past year. It has less sensitivity, but higher specificity, compared to 24-hour dietary recall and dietary diary methods (112). However, it is quick, informative and relatively inexpensive to use in epidemiological studies. The FFQ includes frequency alternatives (from once a month to several times per day), the number of portion eaten and portion sizes (e.g. slices, glasses, cups, pieces, spoons). Therefore, the estimated total energy consumption may depend on the participants' ability to remember what they have eaten over the past year. In addition, participants' willingness to report about their real dietary consumption is also important. For example, obese individuals often under-report their total and individual food consumption (113).

Daily nutrient intakes were computed from a database and software system developed at the Department of Nutrition, University of Oslo (Kostberegnings-SYSTEM, version 3.2). This database also has potential misclassifications in estimation of nutrient intakes merely due to changes in the contents of food and beverages over time. For example, vitamin content of fruit and vegetables may vary according to season, and contents of 'ready to eat' foods may

vary between producers. Moreover, the preparation of food including cooking methods may impact the nutrient content in foods, folate and riboflavin, for example, are susceptible to losses during cooking (114, 115). In addition, our database could not comprehensively assess the impact of vitamin supplement use, which could lead to systematic misclassification of total vitamin consumption.

Blood samples. No instructions were given to participants regarding fasting status before the blood draw, thus most participants were not fasting. Concentrations of plasma tHcy, choline and betaine may be affected by prandial status and reflect recent dietary intake (103, 116). Moderate increase in plasma choline and betaine was observed 2-3 hours after a meal (103). We found that plasma choline and betaine were gradually lower with increasing time since last meal (from 1 to 11 hours), while tHcy was higher, as reported previously (116). However, the decreased concentrations of plasma choline and betaine and the increased level of tHcy may probably not affect the study associations.

One may argue that non-fasting blood samples with accompanying postprandial increases in blood glucose and triglycerides may attenuate the observed relations with choline and betaine. This could be possible due to moderate increase of choline and betaine for 2-3 hours after a meal (103), as was discussed above. However, recent studies suggest that postprandial TG values may be a stronger predictor of CVD risk than fasting levels (117). In addition, we determined non-HDL cholesterol, which is reliable in non-fasting serum and may be used for risk assessment instead of TG and LDL levels, as was suggested by the Third Report of the National Cholesterol Educational Program (NCEP) Adult Treatment Panel III (118).

The influence of factors known to affect plasma tHcy concentration was minimized during

sample collection and storage, by following a strict procedure. Samples (EDTA plasma) were stored at -80° C before analyses. The delay before analyses may have lead to an artificial increase in plasma tHcy concentration by 0.5μmol/L due to additional release of homocysteine from blood cells (102). This release may increase the low values of tHcy and reduce the difference between the highest and the lowest tHcy levels. Thus, tHcy release from blood cells has a propensity to attenuate the effect of tHcy on choline and betaine, as well as on CVD risk factors (119).

In study 1 and 2 we used data on plasma tHcy which was determined by automated HPLC with fluorescence detection. This method has high sensitivity for detection of tHcy. Intra-assay coefficient of variation was 3% (102). However, because a new analytical procedure based on normal-phase chromatography-tandem mass spectrometry (LC-MS/MS) was developed for detection of choline, betaine and several other metabolites, in study 4 we therefore used data on tHcy and creatinine assessed by this method (103). The tHcy values measured by the two methods were highly correlated.

Blood pressure, smoking and physical activity. To get a more stable data on blood pressure, it was measured after 10 min seated resting using Dinamap 845 XT equipment (Criticon, Tampa, Fla) three times and the mean values of three measurements were used for analyses. Because data on smoking and physical activity were collected via self-administered questionnaire, it may lead to misclassifications in estimation of prevalence and intensity of daily smoking and physical activity and systematic errors in our papers. Underestimation of smoking may lead to underestimation of its effect on tHcy, choline and betaine. Therefore, to reduce information bias in study 2, 3 and 4, former smokers were combined with subjects who never smoke and only effect of current smoking vs. non-smoking was evaluated. In

addition, physical activity was categorized in two contrasting groups: no and low vs. medium and high activity, because overestimation of real physical activity may cause overestimation its effect on choline and betaine in study 4.

Confounding

Confounding is a situation in which a third factor may explain (all or part of) the observed association between an exposure and outcome. A confounding factor (confounder) is a risk factor for the outcome (e.g. tHcy, choline and betaine) and it is associated with exposure (e.g. foods, nutrients), but not affected by exposure or disease (110). To reduce the effect of a possible confounder the observed associations should be adjusted for its effect. In our study, we assumed that age and gender might be possible confounders in the associations between predictors and plasma tHcy, choline and betaine. Therefore, we tested all associations in all the four sex and age groups, or adjusted for sex and age group.

Major dietary predictors of plasma tHcy

Because the aim of this study was to determine dietary predictors of plasma tHcy we assumed that the use of vitamin supplements might be a potential confounder. In stratified analysis by user/non-user of vitamin supplements, we observed differences in associations with several food groups and nutrients between non-users and users of vitamin supplements. In addition, considering that B-vitamin supplementation may substantially reduce plasma tHcy concentration and therefore reduce the effects of dietary factors, we excluded users of vitamin supplements for further in-depth analyses. We also adjusted, in multivariate analyses, for known risk factors for plasma tHcy, such as energy intake, smoking, coffee consumption, BMI and creatinine.

To test whether the observed associations between non-B-vitamins and tHcy concentration could be due to confounding effects of dietary B-vitamin intake (73, 74), we repeated the analyses using a model that included adjustment for dietary folate, vitamin B_{12}, B_6 and riboflavin and also for plasma folate and vitamin B_{12}.

Dietary fat as predictors of plasma tHcy

We observed several important differences in associations between tHcy and FAs between younger and older individuals. Therefore, we stratified by age group. For the assessment of tHcy determinants, we considered the following variables to be potential confounders and adjusted for them in the multivariate models: sex, energy intake, daily smoking and coffee intake. We added the following additional variables as potential confounders: intake of vegetables and fruits for the association between other foods and tHcy; and intake of folate and vitamins B_6 and B_{12} and riboflavin for the association between fat types and tHcy. In addition, we stratified individuals by dietary total B-vitamin score when we examined associations between VLC n-3 FAs and tHcy. Moreover, we excluded users of fish-oil and B-vitamin supplements from further analysis.

Predictors of plasma choline and betaine

Because non-fasting samples were analyzed, we adjusted for time since last meal. Inclusion of this variable did however not materially change the observed associations. Because betaine concentration was higher with increasing energy intake, we also adjusted all association for total energy intake.We assumed that lifestyle factors, BMI and creatinine could be potential confounders in the associations between choline, betaine and diet, and therefore adjusted for these.

A potential important confounder could be menopausal status among women, especially in analyses of predictors of plasma choline (50). Unfortunately, we did not have information about the menopausal status of the youngest women. In premenopausal women the concentration of choline is higher then in postmenopausal women, as well as in men, due to *de-novo* synthesis of choline via PEMT. However, our results showed that plasma choline was higher in men than in women and it was higher in older women than in younger. Therefore, we do not think that confounding by menopausal status can explain our findings.

Generalizability

External validity refers to whether results of a study can be generalized to populations outside the study sample. The effect of diet on tHcy, may probably not vary according age and sex, while it may depend on vitamin supplements use. We observed associations between foods, drinks and nutrients, and plasma tHcy, choline and betaine in the same direction for men and women and for older and younger individuals supporting the external validity of our findings. We believe that results reported here are generalizable to Norwegian men and women older than 47 years who do not use vitamin supplements. Our results may not be generalized to vitamin supplement users or populations with mandatory vitamin food fortification. In addition, our results can not be generalized to a population with different dietary habits or younger or ethnically different populations.

The associations between choline and betaine with smoking, physical activity, body fat, blood lipids, glucose and blood pressure were in the same direction for men and women and for older and younger subjects. We believe that our results on components of metabolic syndrome may be generalized to all Norwegian population older than 47 years, as well as to populations with similar diet and lifestyle.

Discussion of specific results and comparison with other studies

Diet

Our findings show that consumption of foods rich in B-vitamins was inversely associated with tHcy level and positively associated with plasma betaine. A diet high in fat and sugar was related to higher plasma tHcy and lower plasma betaine concentrations. Saturated and monounsaturated fats were inversely related to betaine, while VLC n-3 FAs were inversely related to tHcy and positively related to betaine. In addition, intake of high-fiber bread was associated with higher plasma betaine, while alcohol consumption (except beer) was related to lower betaine concentration. Plasma choline was predicted by egg consumption and dietary cholesterol, but not by any identified dietary pattern.

Users and non-users of vitamin supplements. In accordance with previous reports (63, 82, 120, 121), we observed that users and non-users of vitamin supplements differed with respect to lifestyle, dietary habits and plasma vitamin concentrations. Users were more likely to be non-smokers, consume less coffee and generally have a more healthy diet compared to non-users, they also had lower concentrations of plasma tHcy, and higher concentrations of folate and vitamin B12 as compared to non-users. There was an effect modification of vitamin supplement use on the association between several nutrients, food groups, and plasma tHcy. We are not aware that this has been previously reported.

Nutrients. We observed divergent associations between plasma tHcy, choline and betaine and several nutrients. Our findings confirm previous studies reporting inverse associations between dietary intake of folate, vitamin B_{12}, B_6 and riboflavin, and plasma concentration of tHcy (12, 62, 68, 69). Among the four B-vitamins, the individual effect of folate was strongest

(68, 69). In addition to being strongly inversely related to B vitamins, tHcy was also inversely related to vitamins C, E, D, retinol equivalent and beta-carotene, in accordance with previous reports (67, 74). The intake of these vitamins is correlated with the intake of B-vitamins, indicating overlapping dietary sources. Plasma tHcy was also inversely related to complex carbohydrates and fiber.

The strongest positive predictors of plasma betaine were complex carbohydrates, fiber, folate and thiamine. These relations may be explained by the high betaine content of grain products, which are also rich in complex carbohydrates, fiber, folate and thiamine. These findings are consistent with our finding of a positive association between plasma betaine and dietary patterns characterized by high content of high-fiber bread.

While the inverse association between tHcy and fiber was no longer significant after adjustment for dietary B-vitamins, the inverse association with complex carbohydrates remained significant. This association could nevertheless be due to residual confounding from common dietary sources with B-vitamins. Another possible explanation relates to the construction and limitations of the food frequency questionnaire. Choline and betaine are not included in the Norwegian food composition tables, and we could therefore not examine whether the associations could be due to the dietary content of these factors. Some studies (88, 122) as well as the USDA National Nutrient Database for Standard Reference (123) report a high content of these quaternary ammonium compounds in some plant food. Inverse associations between intake and plasma concentrations of choline, betaine and plasma tHcy have been reported in previous studies (29, 57, 72).

Our finding of a strong positive association between SFAs and tHcy concentration is in agreement with findings in a population-based study in Ireland (75). It is possible that saturated fat intake stimulates PC synthesis via PEMT (98, 124, 125), which causes an increase in plasma tHcy concentration (124, 125). Thus more methyl groups may be used for homocysteine remethylation (38, 41, 126).

Previous studies have reported conflicting results on the effect of VLC n-3 FAs on plasma tHcy. Some studies observed an increase or no effect on tHcy concentrations after fish oil or fish powder (76, 78, 79) supplementation. However, other studies report reductions in tHcy in patients supplemented with fish oil (127, 128) and an inverse relationship between plasma tHcy concentration and VLC n-3 FAs concentration in serum phospholipids (74, 77).

The mechanism by which dietary VLC n-3 FAs could affect tHcy has not been elucidated. It is possible that consumption of VLC n-3 FAs decreases the synthesis of PC. Intake of VLC n-3 FAs could stimulate carnitine (a derivative of betaine) formation (129) and BHMT activity (44). It has also been hypothesized that the effect could be due to modulation of gene expression of the enzyme(s) involved in Hcy synthesis (77).

We found that the inverse association between plasma tHcy and VLC n-3 FAs intake was weakened by adjustment for intake of vitamins B_6 and B_{12}. This could be due to confounding introduced by simultaneous consumption of B_6 and B_{12} rich foods: fish, for example, is a source of both VLC n-3 FAs and B-vitamins (101). In addition, our results may also suggest that individuals with high intake of marine oils have other dietary and lifestyle factors which are consistent with lower plasma tHcy concentration. It was suggested that tHcy may be reduced by a combination of B-vitamins and dietary n-3 fatty acids (12). We also found that

the inverse association between tHcy and VLC n-3 FAs intake occurred only at the highest intake level of B-vitamins. This effect was independent of fish-oil and B-vitamin supplement use.

Food groups and food items. As for nutrients, consumption of foods and beverages was divergently associated with plasma tHcy, choline and betaine. In line with previous studies (67, 81), we found inverse associations between plasma tHcy and dietary intake of citrus fruit, orange juice, cruciferous vegetables, spinach and peppers. In addition, we also found inverse relations with apples, carrots, onions, lettuce, cucumbers, tomatoes, and mushrooms.

We observed, as have others, that plasma tHcy is inversely associated with fish intake (74). However, the association was confounded by simultaneous intake of B-vitamins. Fish was a component in a tHcy-lowering diet among elderly subjects (130) and a daily consumption of 0.7 servings of fish in combination with other foods resulted in a significantly lower tHcy as compared to 0.3 servings (86) in a controlled feeding study in adults. However, these studies did not aim to assess the affect of fish as an individual food on tHcy level.

Plasma tHcy was inversely associated with milk, as was previously reported (73, 81). A negative association between plasma tHcy and skim milk intake in adult men was observed by Oshaug et al. among oil platform workers (80). Moreover, skim milk intake was positively associated with choline in our study and intake of milk choline was inversely related to tHcy level in previous reports (72). Choline is a good source of methyl groups and a precursor of betaine. Thus, consumption of dietary choline may explain the inverse relation between milk intake and tHcy level. In addition, formation of PC via PEMT could be decreased due to high content of PC in milk (88).

As for milk, consumption of eggs was inversely related to tHcy level, while it was positively associated with choline concentration. Our observation that plasma choline showed strong association with egg consumption is in accordance with the high content of phosphatidylcholine in eggs. Thus, this finding may confirm our assumption that consumption of choline from diet could decrease the synthesis of PC via PEMT and hence, could reduce homocysteine concentration.

Non-processed meat was inversely associated with tHcy, although the overall food group of meat was not significantly related to tHcy. Non-processed meat is a source of protein, vitamins B_{12}, B_6, riboflavin and choline which all are negatively associated with plasma tHcy (68, 69, 72). In addition, non-processed meat was positively associated with plasma choline because meat is a good source of dietary choline (72). However, the relation was observed only after adjustment for betaine concentration. It could be possible, that betaine is a confounder in the association between meat and choline.

On the other hand, processed meat was positively associated with tHcy level. Processed meat contains less protein and B-vitamins and more fat than non-processed meat. Moreover, saturated fat is positively related to tHcy, and this may partly explain the positive association with processed meat (86).

The intake of sweets and cakes, high in fat and sugar, was associated with high tHcy concentrations, consistent with our finding that the intake of simple carbohydrates (sugar) was negatively associated with dietary folate, vitamin B_{12}, B_6 and riboflavin. Betaine,

however, was inversely associated with sugar products and beverages. Thus, consumption of sugar may increase PC synthesis, and decrease betaine production from choline.

The consumption of medium/high-fiber bread was inversely association with tHcy, but only after adjustment for B-vitamins in diet and plasma. According to previous reports (72) bread as a good source of dietary betaine was inversely associated with plasma tHcy level. In our analyses also high-fiber bread was the strongest predictor of plasma betaine. Thus, the relation between tHcy and bread may be explained by the high betaine content of grain products.

Dietary patterns. Although in this study we did not examine dietary patterns in relation to tHcy, our results are in general agreement with studies that have done so (83-85, 87), namely inverse associations between tHcy and diets rich in fruits, vegetables, fish, meat, milk, whole grain bread and mushrooms (83-85). Dietary patterns high in refined cereals (85), fat (84, 86) and sugar have been associated with higher tHcy levels (87); this is also in agreement with our findings.

To our knowledge this is the first study examining association between dietary patterns and plasma choline and betaine. The most striking findings were different associations of choline and betaine with dietary patterns and total energy intake. Choline was not related to any of the identified dietary patterns. On the contrary, betaine was inversely associated with a 'Western' dietary pattern. While betaine was positively related to total energy consumption, choline was not. Thus, results of the dietary pattern analysis is consistent with our findings that plasma choline could not be influenced by consumption of common foods or diets, while betaine concentration may vary according to dietary contents.

Other risk factors for CVD

Neither choline nor betaine in plasma has previously been studied in relation to CVD risk factors. Recent studies on dietary choline and betaine have not found any association with CVD (32, 33). However, several established CVD risk factors (131), including older age, male gender, smoking, physical inactivity and high tHcy levels are associated with plasma choline and betaine. As interrelated metabolic compounds involved in methionine and lipid metabolisms, choline and betaine show the associations with CVD risk factors in the same direction.

Our observation that plasma choline is higher in men than in women and in older women than in younger women are opposite to what may by expected from animal studies demonstrating stimulation of PEMT and thereby phosphatidylcholine synthesis by estrogens (47, 132), suggesting additional effect from sex hormones on choline metabolism and distribution.

Both plasma choline and betaine were inversely associated with plasma tHcy level, as reported previously (29, 38, 41, 72). Choline showed a positive relation to betaine as the immediate metabolic precursor of betaine (57). The association between choline and betaine was similar to previously reported results (41, 103, 126). In addition, choline was significantly lower in smokers compared to non-smokers, but only in men. The mechanism by which smoking relate to choline metabolism is yet not understood.

Compared to plasma choline, plasma betaine showed a similar but stronger relation to gender and tHcy in the present, as well as in previous studies (38, 41, 57). The strong effect of gender may be explained by transcriptional regulation of human BHMT by estrogen and androgen

(133). In addition, betaine was higher in individuals with higher physical activity, which could be due to healthier lifestyle in these subjects in general and dietary folate intake in particular. Moreover, the relation with physical activity could be explained by increased oxidation rate in mitochondria, discussed below (134, 135).

Components of metabolic syndrome

Despite the close metabolic link between choline and betaine, they show divergent associations with several components of the metabolic syndrome. Because the correlation between choline and betaine is moderate (r=0.37), it is not inconsistent with divergent associations with a third factor, i.e. key element(s) of the metabolic syndrome.

Choline is associated with BMI, percent body fat, waist circumference, HDL cholesterol and TGs in the opposite direction (to betaine) but is not associated with non-HDL cholesterol and blood pressure. The relation between choline and blood lipids are in agreement with the observation that PC supplementation in humans increases TGs, but does not affect cholesterol concentrations (31). Experimental studies in mice show that high-fat feeding leads to weight gain, elevated triglycerides, hyperinsulinemia and glucose intolerance in choline-replete animals, whereas choline-deficiency increases TG stores in liver (53).

Betaine is inversely associated with several components of the metabolic syndrome (93) in the direction that decreases CVD risk. These factors include BMI, percent body fat, waist circumference, systolic and diastolic blood pressure, non-HDL cholesterol, HDL cholesterol and TGs. These observations gain some support from results published by others. In subjects attending a lipid clinic, betaine was inversely related to body fat and Apo B (44). Betaine supplementation has been shown to reverse an atherogenic lipid profile in mice (136) and to

decrease body fat in pigs (137, 138). However, in humans, high doses of 4-6 g betaine daily increased total cholesterol, LDL cholesterol and TGs (31, 39). It is possible that endogenous and dietary betaine may have different effects on lipid metabolism, and that the effects of betaine supplementation may vary according to dose, duration and also between species.

Diet and the metabolic syndrome. Conceivably, the relations of plasma free choline and betaine to components of the metabolic syndrome may reflect the influence of dietary patterns and recent intake of specific food items. We observed a weak inverse association between choline and betaine concentrations and time since last meal, but adjustment for this time interval did not materially affect the results. Furthermore, energy intake showed no relation to plasma choline but a positive relation to plasma betaine.

The metabolic syndrome has been linked to dietary intake. Although somewhat inconsistent results have been obtained, the prevailing view is that a "Healthy" or prudent diet, with high amounts of fruits, vegetables, legumes, whole grain, poultry, and low-fat dairy products is beneficial. An energy dense 'Western' diet rich in refined grains, cakes, sugar and red meat, fried food and butter is adversely associated with the metabolic syndrome (139-141). However, food items rich in choline (88) such as eggs, low-fat meat and milk, are not necessarily components of an unhealthy dietary pattern. For example, in healthy adults, there was an inverse relation between choline intake and concentrations of inflammatory markers related to atherogenesis (94). Furthermore, high fat dairy products, cakes and sweets are low in choline. Likewise, the betaine content in most fruits and vegetable (except beets and spinach) is low (88).

Because wheat bran and germ are good sources of betaine (88), it could be argued that the

inverse association between plasma betaine and component of the metabolic syndrome is attributable to the consumption of whole wheat. However, even though the interindividual variation of plasma betaine is substantial (up to 10-fold differences between individuals), the intraindividual variability is small, with an individual set point that remains stable for years (142). This suggests that plasma betaine is under strict metabolic control and justifies the concept of betaine status as a component of an individual's homeostasis.

Conceivably, the observed divergent relations of plasma choline and betaine with components of the metabolic syndrome could reflect diet if high intakes of meat increase plasma choline and high intakes of vegetables increase betaine. However, this possibility is not in agreement with the observations that neither plasma choline nor betaine was related to the 'Meat' or 'Vegetables' dietary patterns. Furthermore, cholesterol, probably derived from intake of eggs, was the only nutrient associated with plasma choline, which showed no association with serum non-HDL cholesterol. These observations support the contention that the link between plasma choline and betaine and the metabolic syndrome may not reflect dietary intake.

*Possible mechanisms.*Central obesity and increased biologic activity of the upper visceral adipose tissue with excessive flux of fatty acids are regarded as primary factors of the metabolic syndrome, leading to insulin resistance and atherogenic dyslipidemia (93). Both BHMT and choline dehydrogenase in rat liver are decreased by insulin and increased in diabetes (143). In addition, a general mitochondrial dysfunction prevails in the metabolic syndrome (144), which may involve choline oxidation to betaine (145). Thus, the metabolic syndrome including insulin resistance may be associated with a disruption of the choline dehydrogenase pathway in the motochondria. Moreover, this disruption could be possible when excessive flux of fatty acids requires choline for PC synthesis (98) and VLDL secretion

(146).

Recent studies suggest a role of BHMT in lipid metabolism (147). Dietary BHMT induction in rats resulted in an increase in liver apoprotein, TGs production and VLDL secretion (147). These are features suggesting roles in addition to homocysteine remethylation for this enzyme (133). Thus, BHMT may represent a metabolic link between lipid and methionine metabolism.

6 CONCLUSIONS

Our findings from this Norwegian adult and elderly population sample indicate that diet influences the concentrations of plasma betaine and tHcy. A diet rich in B-vitamins and with a low content of fat and sugar is related to higher plasma betaine and lower tHcy levels.
In a population not taking vitamin supplements, plasma tHcy concentration may be lowered with a diet rich in complex carbohydrates, protein, and B-vitamins. Such a diet includes vegetables, fruits, whole grain bread and cereals, as well as fish, non-processed meat, chicken, and eggs. Moreover, a diet rich in complex carbohydrates, fiber, folate and vitamin B_6 is related to higher plasma betaine level. Plasma choline was less influenced by diet, and was only positively associated with eggs, skim milk and beer.

On the other hand, a high consumption of dairy fat and sugar-rich foods may decrease plasma betaine and increase tHcy concentration. In addition, the inverse association between dietary intake of VLC n-3 FAs and plasma tHcy may be explained by the positive relation of betaine to VLC n-3 FAs and by simultaneous consumption of B-vitamins from the same food source.

In addition, the present study demonstrates that key components of the metabolic syndrome including body composition, blood lipids and glucose are related to plasma choline and betaine. An increased CVD risk factor profile is associated with low betaine and high choline levels. These divergent associations of the substrate (choline) and product (betaine) may reflect disruption of the mitochondrial choline dehydrogenase pathway. Low choline oxidation may be caused by a diet high in saturated fat and sugar. Thus, a Western style diet leading to decreased choline oxidation may influence the concentration of both betaine and tHcy in plasma and hence, increase the risk for CVD.

7 FUTURE STUDIES

Future studies should examine betaine content in common foods and beverages in order to evaluate total consumption of dietary betaine and its effect on betaine and tHcy in plasma. Moreover, whether this effect is independent of dietary B-vitamins also should be evaluated. In addition, consumption of dietary choline (62, 72) should be assessed in relation to plasma choline and tHcy levels.

To confirm the relation between dietary patterns and plasma betaine, more studies should examine the affect of 'healthy' and 'unhealthy' dietary patterns on plasma betaine level using confirmatory factor analysis.

More large observational and intervention studies are needed to confirm our findings on association between VLC n-3 FAs intake and plasma betaine and tHcy. In addition, future studies should assess an effect of saturated fat and sugar/glucose on plasma choline, betaine and tHcy levels.

Further investigations should confirm the relations of CVD risk factors and key components of the metabolic syndrome (body composition, lipid profile, glucose and blood pressure) to plasma choline and betaine levels. Moreover, choline and betaine levels should be evaluated in relation to non-alcoholic fatty liver disease.

To confirm an assumption that the link between plasma choline and betaine and the metabolic syndrome may not reflect merely dietary intake of choline or betaine, but rather a disruption of mitochondrial choline dehydrogenase pathway (145) by FAs, the affect of different diets (low SFAs vs. high SFAs and low VLC n-3 FAs vs. VLC n-3 FAs) on choline dehydrogenase

activity should be tested. In addition, the general mitochondrial dysfunction that prevails in the metabolic syndrome (144), should be examined in response to different diets in obese and healthy subjects.

More studies on humans should be done to test our assumption that plasma choline and betaine concentrations may vary according enzyme activities, BHMT (133) and choline dehydrogenase (145), as well as of PC synthesis (148, 149). Effect of insulin on the enzymes activity should be assessed in diabetes and healthy humans (143).

Intervention studies are needed to test the inverse relations between plasma betaine and alcohol and between choline and coffee consumption. In addition, choline and betaine level should be evaluated in relation to kidney function.

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9 APPENDICES

APPENDIX I

Questionnaire

HELSEUNDERSØKELSEN I HORDALAND 1997-99

☐ Adresse endring

Personlig innbydelse

SP02B

Spørreskjemaet er en viktig del av helseundersøkelsen. Vennligst fyll ut skjemaet på forhånd og ta det med til helseundersøkelsen. Dersom enkelte spørsmål er uklare, lar du dem stå ubesvart til du møter fram, og drøfter dem med personalet som gjennomfører undersøkelsen.
Alle svar vil bli behandlet strengt fortrolig.

Det utfylte skjemaet vil bli lest av en maskin. Bruk blå eller sort farge ved utfylling. Det er viktig at du går fram slik:

- i de små boksene setter du kryss for det svaret som passer best for deg
- i de store boksene skriver du tall eller blokkbokstaver – NB! innenfor rammen for boksen.

Eksempler:
Avkryssing: ☒ Tall: 1 2 3 4 5 6 7 8 9 0 Bokstaver: A B C

Med vennlig hilsen

Statens helseundersøkelser ♥ Kommunehelsetjenesten ♥ Helseundersøkelsen i Hordaland

1. EGEN HELSE

Hvordan er helsen din nå? *(Sett bare ett kryss)*

Dårlig	Ikke helt god	God	Svært god
☐ 1	☐ 2	☐ 3	☐ 4

Har du, eller har du hatt:	JA	NEI	Alder første gang
Hjerteinfarkt	☐	☐	år
Angina pectoris (hjertekrampe)	☐	☐	år
Hjerneslag/hjerneblødning	☐	☐	år
Astma	☐	☐	år
Diabetes (sukkersyke)	☐	☐	år
Multippel sklerose	☐	☐	år

Bruker du medisin mot høyt blodtrykk?

Nå	Før, men ikke nå	Aldri brukt
☐ 1	☐ 2	☐ 3

Har du noen gang det siste året hatt eksem (rød, kløende, sår og sprukken hud):	JA	NEI
På hendene?	☐	☐
I ansiktet?	☐	☐
Andre steder på kroppen?	☐	☐

	JA	NEI
Med «hvite fingre» mener vi plager i form av at en eller flere fingre blir hvite og at man samtidig mister følelsen i dem når det er kaldt. Har du slike plager?	☐	☐

2. HVORDAN FØLER DU DEG?

Har du de siste to ukene følt deg:

	Nei	Litt	En god del	Svært mye
Nervøs og urolig?	☐	☐	☐	☐
Plaget av angst?	☐	☐	☐	☐
Trygg og rolig?	☐	☐	☐	☐
Irritabel?	☐	☐	☐	☐
Glad og optimistisk?	☐	☐	☐	☐
Nedfor/deprimert?	☐	☐	☐	☐
Ensom?	☐ 1	☐ 2	☐ 3	☐ 4

3. SYKDOM I FAMILIEN

	JA	NEI	VET IKKE
Har en eller flere av foreldre eller søsken hatt hjerteinfarkt (sår på hjertet) eller angina pectoris (hjertekrampe)?	☐	☐	☐
Har en eller flere foreldre/søsken hatt:			
Hjerteinfarkt *før* de fylte 60 år?	☐	☐	☐
Hjerneslag/hjerneblødning *før* de fylte 70 år?	☐	☐	☐

4. MUSKEL- OG SKJELETTPLAGER

	JA	NEI
Har du i løpet av det siste året vært plaget med smerter og/eller stivhet i muskler og ledd som har vart i minst 3 måneder sammenhengende?	☐	☐

Hvis NEI, gå til avsnitt 5.
Hvis JA, svar på følgende:

Hvor har du hatt disse plagene?	JA	NEI
Nakke	☐	☐
Skuldre (aksler)	☐	☐
Albuer	☐	☐
Håndledd/hender	☐	☐
Bryst, mage	☐	☐
Øvre del av ryggen	☐	☐
Korsryggen	☐	☐
Hofter	☐	☐
Knær	☐	☐
Ankler, føtter	☐	☐

Hvor lenge har plagene vært sammenhengende?
Svar for det området hvor plagene har vært lengst.

Hvis under 1 år, oppgi antall måneder..... *Antall mnd.* ☐☐
Hvis 1 år eller mer, oppgi antall år ..... *Antall år* ☐☐

Har plagene redusert din arbeidsevne det siste året?
Gjelder også hjemmearbeidende. Sett bare ett kryss.

Nei/ubetydelig	I noen grad	I betydelig grad	Vet ikke
☐ 1	☐ 2	☐ 3	☐ 4

	JA	NEI	Ikke i arbeid
Har du vært sykmeldt p.g.a. disse plagene det siste året?	☐	☐	☐

	JA	NEI
Har plagene ført til redusert aktivitet i fritiden?	☐	☐

5. MOSJON

Hvordan har din fysiske aktivitet i fritiden vært det siste året?
Tenk deg et ukentlig gjennomsnitt for året.
Arbeidsvei regnes som fritid. Besvar begge spørsmålene.

	Timer pr. uke: Ingen	Under 1	1-2	3 og mer
Lett aktivitet *(ikke svett/andpusten)*	☐	☐	☐	☐
Hard fysisk aktivitet *(svett/andpusten)*	☐ 1	☐ 2	☐ 3	☐ 4

6. KAFFE / TE / ALKOHOL

Hvor mange kopper kaffe/te drikker du daglig?
Sett 0 hvis du ikke drikker kaffe/te daglig.

Antall kopper daglig

Kokekaffe	Annen kaffe	Te

	JA	NEI
Er du total avholdsmann/-kvinne?	☐	☐

Hvor mange ganger i måneden drikker du vanligvis alkohol? *Regn ikke med lettøl.*
Sett 0 hvis mindre enn 1 gang i mnd. ..... *Antall ganger*

Hvor mange glass øl, vin eller brennevin drikker du VANLIGVIS i løpet av to uker?
Regn ikke med lettøl. Sett 0 hvis du ikke drikker alkohol.

Glass øl	Glass vin	Glass brennevin

7. RØYKING

Hvor lenge er du vanligvis daglig tilstede i røykfylt rom? ..... *Antall hele timer*
Sett 0 hvis du ikke oppholder deg i røykfylt rom.

Røyker du selv:	JA	NEI
Sigaretter daglig?	☐	☐
Sigarer/sigarillos daglig?	☐	☐
Pipe daglig?	☐	☐

Aldri røykt daglig ..... (Sett kryss) ☐

Hvis du har røykt daglig tidligere, hvor lenge er det siden du sluttet? ..... *Antall år*

Hvis du røyker daglig nå eller har røykt tidligere:

Hvor mange sigaretter røyker eller røykte du vanligvis daglig? ..... *Antall sigaretter*

Hvor gammel var du da du begynte å røyke daglig? ..... *Alder i år*

Hvor mange år til sammen har du røykt daglig? ..... *Antall år*

8. ENDRING AV HELSEVANER

Dette gjelder din interesse for å endre helsevaner. Røykespørsmålet besvares bare av dem som røyker.

	Spise sunnere		Trimme mer		Slutte å røyke	
	JA	NEI	JA	NEI	JA	NEI
Har du de siste 12 mnd. forsøkt å:	☐	☐	☐	☐	☐	☐
Om 5 år, tror du at du har endret vaner på noen av disse områdene?	☐	☐	☐	☐	☐	☐

	Høyeste vekt	Laveste vekt
Anslå din høyeste og laveste vekt i løpet av de siste 5 år. *(Hele kg)* *(Se bort fra vekt under svangerskap)*		

9. UTDANNING

Hvilken utdanning er den høyeste du har fullført?
Sett bare ett kryss.

Mindre enn 7 år grunnskole	☐
Grunnskole 7-10 år, framhaldsskole, folkehøgskole	☐ 1
Realskole, middelskole, yrkesskole, 1-2 årig videregående skole	☐ 2
Artium, øk.gymnas, allmennfaglig retning i videregående skole	☐ 3
Høgskole/universitet, mindre enn 4 år	☐ 4
Høgskole/universitet, 4 år eller mer	☐ 5

10. HELSE OG TRIVSEL

De neste spørsmålene handler om hvordan du ser på din egen helse. Hvis du er usikker på hva du skal svare, vennligst svar så godt du kan.

Er din helse slik at den begrenser deg i utførelsen av disse aktivitetene NÅ?

Moderate aktiviteter som å flytte bord, støvsuge, gå en tur eller drive med hagearbeid:

Ja, begrenser meg mye	Ja, begrenser meg litt	Nei, begrenser meg ikke i det hele tatt
☐	☐	☐

Gå opp trappen flere etasjer:

Ja, begrenser meg mye	Ja, begrenser meg litt	Nei, begrenser meg ikke i det hele tatt
☐	☐	☐

I løpet av de siste 4 ukene, har du hatt noen av de følgende problemer i ditt arbeid eller i andre av dine daglige gjøremål på grunn av din fysiske helse?	JA	NEI
Du har utrettet mindre enn du hadde ønsket	☐	☐
Du har vært hindret i å utføre visse typer arbeid eller gjøremål	☐	☐

I løpet av de siste 4 ukene, har du hatt noen av de følgende problemer i ditt arbeid eller i andre av dine daglige gjøremål p.g.a. følelsesmessige problemer? *(Som f.eks. å være deprimert eller engstelig)*	JA	NEI
Du har utrettet mindre enn du hadde ønsket	☐	☐
Du har utført arbeidet eller andre gjøremål mindre grundig enn vanlig	☐	☐

I løpet av de siste 4 ukene, hvor mye har smerter påvirket ditt vanlige arbeid?
(Gjelder både i og utenfor hjemmet) Sett bare ett kryss.

	JA
Ikke i det hele tatt	☐ 1
Litt	☐ 2
En del	☐ 3
Mye	☐ 4
Svært mye	☐ 5

Hvor ofte i løpet av de siste 4 ukene har du følt deg rolig og harmonisk? Sett bare ett kryss.

	JA
Hele tiden	☐ 1
Nesten hele tiden	☐ 2
Mye av tiden	☐ 3
En del av tiden	☐ 4
Litt av tiden	☐ 5
Ikke i det hele tatt	☐ 6

Hvor ofte i løpet av de siste 4 ukene har du hatt mye overskudd? Sett bare ett kryss.

JA

- Hele tiden ☐ 1
- Nesten hele tiden ☐ 2
- Mye av tiden ☐ 3
- En del av tiden ☐ 4
- Litt av tiden ☐ 5
- Ikke i det hele tatt ☐ 6

Hvor ofte i løpet av de siste 4 ukene har du følt deg nedfor og trist? Sett bare ett kryss.

JA

- Hele tiden ☐ 1
- Nesten hele tiden ☐ 2
- Mye av tiden ☐ 3
- En del av tiden ☐ 4
- Litt av tiden ☐ 5
- Ikke i det hele tatt ☐ 6

I løpet av de siste 4 ukene, hvor mye av tiden har din fysiske helse eller følelsesmessige problemer påvirket din sosiale omgang(som det å besøke venner, slekt)? Sett bare ett kryss.

JA

- Hele tiden ☐ 1
- Nesten hele tiden ☐ 2
- Mye av tiden ☐ 3
- En del av tiden ☐ 4
- Litt av tiden ☐ 5
- Ikke i det hele tatt ☐ 6

Stort sett, vil du si at din helse er:

Utmerket	Meget god	God	Nokså god	Dårlig
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

11. BRUK AV MEDISINER

Med medisiner mener vi her alle slags medisiner, både:
- *med og uten resept, naturmedisin, vitaminer og mineraler*
- *medisin som svelges, inhaleres eller injiseres, stikkpiller, salver, kremer eller dråper.*

Tok du noen slags medisiner I GÅR? JA ☐ NEI ☐

Hvis NEI, kan du gå til avsnitt 12.

Hvis JA, besvar følgende:

Hvilke medisiner tok du I GÅR, og hva var grunnen til at du tok medisinen (diagnose, sykdom, symtom, helseeffekt)?

Sett svarene inn i skjemaet nedenfor, en linje for hver medisin. Kryss av for ja om du bruker medisinen daglig eller nesten daglig.

Navn på medisinen (ett navn pr. linje):	Grunn til bruk av medisinen I GÅR var:	Daglig JA	Daglig NEI

Dersom det ikke er nok plass her, kan du fortsette på eget ark som legges ved.

12. ARBEID

Besvares av dem som har hatt inntektsgivende arbeid i minst 100 timer det siste året.

Beskriv virksomheten på det arbeidsstedet der du utførte inntektsgivende arbeid i lengst tid de siste 12 mnd. *(Skriv f.eks. jordbruk, barneavd. på sykehus, snekkeravd. på skipsverft e.l.).*

Virksomhet: ______

Hvilket yrke/tittel har eller hadde du på dette arbeidsstedet? *(Skriv f.eks. kornbonde, anestesisykepleier, snekker e.l.)*

Yrke: ______

Hvor lenge har du praktisert i dette yrket i ditt liv? *Antall år i yrket* ☐☐

Har du noen av de følgende yrker (*heltid eller deltid*)? *Sett kryss for hvert spørsmål.*

	JA	NEI
Sjåfør	☐	☐
Bonde/gårdbruker	☐	☐
Fisker	☐	☐

Har du tidligere i ditt liv (*ikke i dag*) hatt inntektsgivende arbeid som:

	JA	NEI
Bilmekaniker/biloppretter	☐	☐
Frisør	☐	☐

13. SAMLIV

Oppgi antall egne barn (eventuelt 0) av hvert kjønn:

Antall gutter ☐☐ *Antall jenter* ☐☐

Har du noen gang hatt regelmessig samliv uten prevensjon i ett år eller mer uten at det har ført til graviditet? JA ☐ NEI ☐

Med prevensjon menes også mer usikre metoder som avbrutt samleie, «sikre perioder» etc.

De følgende spørsmål besvares bare av kvinner

Har du noen gang spontanabortert (ufrivillig mistet fosteret) etter at graviditet var sikkert påvist?

NEI ☐ USIKKER ☐ JA ☐ **Hvis JA:** *Antall ganger* ☐☐

Følgende spørsmål besvares bare hvis du har vært gravid:

Oppgi antall måneder det tok med regelmessig samliv uten prevensjon (eller evt. amming), til du ble gravid:

- Siste svangerskap ☐☐ *mnd. uten prevensjon*
- Nest siste svangerskap ☐☐ *mnd. uten prevensjon*
- Tredje siste svangerskap ☐☐ *mnd. uten prevensjon*

14. ETTERUNDERSØKELSE

Hvis denne helseundersøkelsen viser at du bør undersøkes nærmere, hvilken allmennpraktiserende lege/kommunelege ønsker

______

Ikke skriv i disse rutene ☐☐☐☐ ☐☐☐

Takk for utfyllingen!
Nå en gang: ***Velkommen til undersøkelsen***

IE 3215801 (IDNR. 1/97) - 30000 - Beyer-Hecos 8.97

APPENDIX II

Informed consent
Information sheet

HORDALANDSUNDERSØKELSEN '97 - '99 (HUSK)

SAMTYKKEERKLÆRING

I brosjyren "HUSK" er jeg orientert om Hordalands-undersøkelsens formål. Jeg har også sett informasjonsskrivet "HUSK INFO" som bl.a. omtaler delprosjekter, og er kjent med at undersøkelsen består av spørreskjema, blodprøve og måling av blodtrykk, høyde, vekt, liv- og hoftevidde.

Jeg er kjent med at opplysninger om meg blir behandlet strengt fortrolig og at undersøkelsen er vurdert og tilrådd av Den regionale komité for medisinsk forskningsetikk og godkjent av Datatilsynet. Det er ikke satt noen spesiell tidsbegrensning for hvor lenge opplysningene kan lagres, men jeg er klar over at jeg på hvilket som helst tidspunkt kan trekke meg fra undersøkelsen og kan reservere meg mot bruk av opplysninger om meg.

1. Jeg samtykker i at resultater fra blodprøven og andre deler av undersøkelsen, samt resultater fra eventuelle spesialundersøkelser, blir sendt til den legen jeg har oppgitt på spørreskjemaet.

2. Dersom jeg ikke har oppgitt navn på lege, eller legen min ikke deltar i undersøkelsen, samtykker jeg i at mine resultater sendes til kommunelege I.

3. Jeg samtykker i at jeg kan få tilbud om spesialundersøkelser, og at jeg kan bli kontaktet av en lege med tanke på tilbud om behandling eller for å forebygge sykdom.

4. Jeg samtykker i at mine resultater kan brukes til medisinsk forskning, eventuelt ved å sammenholde opplysninger om meg med opplysninger fra andre helse-, trygde- og sykdomsregistre, eller med mine resultater fra tidligere helseundersøkelser i Hordaland. Når disse opplysningene sammenholdes, vil mitt navn og personnummer ikke bli tatt med.

5. Jeg samtykker i at blodprøve oppbevares. All bruk av denne vil bare skje etter godkjenning fra Datatilsynet og Den regional komité for medisinsk forskningsetikk.

Vennligst stryk det/de avsnitt du reserverer deg mot.

....

Sted og dato Underskrift

FRAMMØTE DATO

Sykepl. kode BT 84

DAG MND. ÅR 17

HØYDE

VEKT

H/V 25

ANM.

AVVIK BT-MÅL.

ARM OMKR.

MANSJ. STØRR.

AP.NR

TSM 32

KOMMUNE 85-88

MÅLING 1

MAP 35

S 38

HR 53

D 56

MÅLING 2

MAP 41

S 44

HR 59

D 62

MÅLING 3

MAP 47

S 50

HR 65

D 68

SAMTYKKE
Tilhørende hjerte-kar.

JA/NEI

1 2 3 4 5 74

SAMTYKKE
Kvinneutvalg

JA/NEI 75

OMKRETS

MIDJE 78

HOFTE 81

Sykepl. kode blod-prøve

(ID-1/97) 30.000. 10-97. e.s.trykk

HUSK INFO

for fødselsårene 1925, 26, 27 og 1950, 51

Takk for at du deltok i den forrige Hordalandsundersøkelsen i 1992/93, og at du nå deltar i den nye Helseundersøkelsen i Hordaland, som har fått navnet HUSK. Den forrige undersøkelsen fikk frem mye verdifull informasjon, og satte for alvor Bergen og Hordaland på verdenskartet for forskning rundt kroniske sykdommer. Spesielt viktig var målingen av en ny risikofaktor for hjertekarsykdom: homocystein i blodet (se nedenfor).

Første fase av HUSK er den undersøkelsen du nå får tilbud om å delta i. Andre fase består i oppfølging av enkelte utvalg både på kort og lang sikt. Det er ikke satt noen spesiell tidsbegrensning for hvor lenge opplysningene kan lagres. Dette er fordi det i fremtiden kan komme ny informasjon som gjør at en vil belyse andre sider av helse og sykdom enn det som er kjent i dag. Fremtidig kobling til andre helseregistre vil kun skje etter godkjenning fra Datatilsynet, og slik at ingen opplysninger kan føres tilbake til den enkelte. Opplysningene fra de ulike delene av undersøkelsen vil kunne bli sammenstilt med hverandre, f.eks. vil en kunne se om de som har god sosial støtte har lavere blodtrykk eller blodkolesterol enn de med mindre god sosial støtte, eller om personer som har vært eller er i bestemte yrker er mer utsatt for eksem enn personer i andre yrker.

Ansvarlige for undersøkelsen - Styringsorganer

En styringsgruppe bestående av representanter for Statens Helseundersøkelser, HEMIL-senteret og Institutt for Samfunnsmedisinske fag ved Universitetet i Bergen er ansvarlig for HUSK. Fylkeslegen i Hordaland er observatør til denne gruppen. Koordineringen og den daglige styringen av prosjektet foregår fra Seksjon for forebyggende medisin, Universitetet i Bergen.

Økonomisk støtte

Til administrasjon og gjennomføring av delprosjekter er vi tildelt midler fra Norges forskningsråd, Sosial- og helsedepartementet, Kommunal- og arbeidsdepartementet, Det medisinske fakultet ved Universitetet i Bergen, L. Meltzers høyskolefond, Rådet for psykisk helse, Den Norske Kreftforening og Norsk osteoporoseforening. Vi er også tildelt støtte fra NHOs Arbeidsmiljøfond, Kavlifondet, Dr. Trygve Gythfeldt og frues forskningsfond, Røde fjær-aksjonen og farmasøytisk industri. Vi takker alle våre bidragsytere.

DELPROSJEKTER

- **Homocystein** i blodet er en ny risikofaktor for hjerte- og karsykdom. Den forrige undersøkelsen i 1992/93 gav oss verdifulle resultater. Blant annet ble det vist at noen forhold som kan føre til høyt homocysteinnivå i blodet er røyking, høyt kaffeinntak, lite mosjon og lavt inntak av frukt og grønnsaker. Andre studier tyder på at homocystein kan være en like viktig faktor som kolesterol med hensyn til risiko for hjerte- og karsykdom.

Du inviteres nå til en ny homocystein-undersøkelse. Denne tar sikte på å studere sammenhenger mellom kosthold, B-vitaminstatus og faktorer som påvirker homocysteinnivået, samt å kartlegge faktorer av betydning for endringer av homocystein. Videre vil variasjoner for gener som har betydning for omsetningen av B-vitaminer i kroppen bestemmes. Disse genene er ikke årsak til sykdom, men de kan sannsynligvis bidra til å forklare hvorfor forskjellige mennesker har ulikt behov for B-vitaminer. Disse analysene vil være av stor verdi fordi vi kan da finne ut om noen mennesker kan ha særlig nytte av et B-vitaminrikt kosthold, eller tilskudd av B-vitaminer. Blodprøven som lagres vil senere kunne undersøkes for andre faktorer knyttet til kroppens omsetning av B-vitaminer. For å måle kostens innhold av B-vitaminer og andre faktorer, ber vi deg om å fylle ut et spørreskjema om dine kostvaner, samt et skjema som bl.a. omhandler eventuell sykdom siden forrige undersøkelse. Personer med sterkt forhøyet homocystein og tegn på alvorlig mangel på B-vitaminene folat eller B-12, vil få tilbud om etterundersøkelse. For de fleste deltakerne vil det ikke være noen unormale funn, og det vil ikke bli gitt tilbakemelding.

- **Muskel-skjelett sykdommer**. Dere vil også få et tilbud om målinger av benmineraltetthet som mål på osteoporose (benskjørhet). Osteoporose er en tilstand som rammer svært mange kvinner etter som de blir eldre, og også en del menn. Den alvorligste følgen av osteoporose er benbrudd. I dette prosjektet vil sammenhengen mellom mengde mineraler i skjelettet og andre faktorer som påvirker osteoporose belyses blant annet vil kroppssammensetningen, som prosent fett, måles. Målingene vil foregå på Haukeland sykehus og er helt ufarlige.

- Det vil også gjøres en studie på forekomsten av **Sjögrens syndrom**, som er en reumatisk lidelse, med kartlegging av forskjellige faktorers betydning for denne tilstanden.

- **Hukommelse og minne.** Dette prosjektet gjelder kun for de som er født i 1925-27. Mye tyder på at homocystein og B-vitaminer i kosten kan ha betydning for hukommelse og minne etter som vi blir eldre. Gjennom et intervju med en sykepleier/hjelpepleier vil deltakernes hukommelse, minne, orienteringsevne og forståelsesevne bli belyst.

- **Lungefunksjonstest (spirometri).** Her vil deltakernes lungekapasitet bli målt ved en pusteprøve, og forekomsten av astma vil bli kartlagt.

- **Arbeidsrelaterte plager i Hordaland**. Som følge av liten kunnskap om arbeidsrelaterte plager i Norge tas ofte avgjørelser m.h.t. forebyggende tiltak på tildels manglende grunnlag. Dette prosjektet vil bl.a. undersøke forekomsten av yrkesrelatert hjerte-karsykdom, muskel-skjelettplager, vibrasjonsskader, håndeksem og forplantning.

- **Subjektiv helse, livskvalitet og søvn** skal kartlegge forekomst av og sammenheng mellom helseplager og livskvalitet, som for eksempel søvnplager.

- **Sosiale prosesser.** Her vil man forsøke å identifisere hvilke sosiale prosesser som påvirker helsen. **Angst og depresjon** vil også bli belyst.

- **Hemokromatose** er en arvelig stoffskiftesykdom som fører til jernoverskudd som kan gi organskade og sykdom. Undersøkelsen tar sikte på å kartlegge forekomst, og dernest å identifisere behandlingstrengende personer for videre oppfølging.

husk

APPENDIX III

Food frequency questionnaire

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)	☐	☐	☐	☐
												1/2	1	1 1/2	2+
Lettyoghurt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(beger)	☐	☐	☐	☐
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	☐	☐	☐	☐	☐	☐
Brus, Cola, Solo o.l., kunstig søtet	☐	☐	☐	☐	☐	☐
Farris, Selters, Soda o.l.	☐	☐	☐	☐	☐	☐
Alkoholfritt øl, vørterøl, lettøl	☐	☐	☐	☐	☐	☐
Pilsnerøl	☐	☐	☐	☐	☐	☐
Vin	☐	☐	☐	☐	☐	☐
Brennevin, likør	☐	☐	☐	☐	☐	☐

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

	1	2	3	4	5	6+
Vin	☐	☐	☐	☐	☐	☐
Brennevin, likør	☐	☐	☐	☐	☐	☐

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+		1-2	3-4	5-6	7-8	9+
Risgrøt, annen melkegrøt	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐	☐
											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	Oljer
☐ Smør (meierismør)	☐ Olivenolje
☐ Bremykt	☐ Soyaolje
☐ Melange, Per	☐ Maisolje
☐ Soft-, soyamargarin (pakke, beger)	☐ Solsikkeolje
☐ Solsikke	☐ Valnøttolje
☐ Oliven	☐ Andre oljer
☐ Annen margarin	

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			
										1 ts	1 bs	1 ss	
Tran	☐	☐	☐	☐	☐	☐	☐	☐		☐	☐	☐	
										1	2+		
Trankapsler	☐	☐	☐	☐	☐	☐	☐	☐	kapsler	☐	☐		
										1-2	3-4	5-6	7+
Fiskeoljekapsler	☐	☐	☐	☐	☐	☐	☐	☐	kapsler	☐	☐	☐	☐
Multipreparater													
			0	<1	1	2-3	4-5	6-7		1	2	3	4+
Sanasol	☐	☐	☐	☐	☐	☐	☐	☐	bs	☐	☐	☐	☐
Biovit	☐	☐	☐	☐	☐	☐	☐	☐	bs	☐	☐	☐	☐
Vitaplex	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Kostpluss	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Vitamineral	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Annet	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐

Hvis annet, hvilket? .....

Jernpreparater													
			0	<1	1	2-3	4-5	6-7		1	2	3	4+
Ferro C	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Hemofer	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Duroferon Duretter	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Annet	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐

Hvis annet, hvilket? .....

			0	<1	1	2-3	4-5	6-7		1	2	3	4+
B-vitaminer	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
C-vitamin	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
D-vitamin	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
E-vitamin	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Folat (folsyre)	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
			0	<1	1	2-3	4-5	6-7		1	2	3	4+
Kalktabletter	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Fluortabletter	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Annet	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐

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!