Self-reported choline intake and associations with circulating parameters of one-carbon and lipid metabolism in a healthy Norwegian population

Master's thesis in Clinical Nutrition

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2021

Preface

Looking back at the past year working with this thesis, I am grateful for the experience and for all the knowledge it has provided me of. It has been different and challenging, but definitely one of the most educational years of my studies at the University of Bergen.

First of all, I would like to thank my supervisor Anthea Van Parys. You have been a great support through this process and guided me in the right direction when I needed your advice. Your knowledge in this field has been to a tremendous help. Thank you for all the support, guidance and encouraging feedback on my work! I would also like to thank co-supervisor Ottar Nygård for your feedback.

It would be a lie to say that my past year has not been affected by Covid-19. Social distancing due to the pandemic has made working with this thesis very independent, and also lonely from time to time. In that context, I would like to thank Helene Dahl and Anthea Van Parys for scheduling a course in R-studio for me and my co-students. It has been extremely helpful in the process of learning R, and to be able to work independently and with a greater understanding of the statistical program. Additionally, it has been a lot of fun and a nice way to keep some contact with friends and classmates.

Additionally, I would like to thank the participants and study personnel working with the HUSK study project. Your work and contribution to the project has been highly appreciated.

Finally, I would like to thank my family, friends and Simon. You have all been a huge support and showed great interest for my work. Thank you for your patience and uplifting words, it would not have been such a great year without you!

Maria Sandvik Brække

Bergen, May 2021

Abstract

Introduction: Choline is an essential nutrient even though it to some extent can be endogenously synthesized in the liver. It is required for the synthesis of phospholipids in cell membranes and for optimal transmembrane signaling, as a central part of the neurotransmitter acetylcholine, and it is closely related to the one-carbon metabolism through its metabolite betaine. In food and in the body, choline occurs in water-soluble forms, including free choline (FC), phosphocholine (PC) and glycerophosphocholine (GC), and in the lipid-soluble forms phosphatidylcholine (PtdCho) and sphingomyelin (SM). PtdCho connects choline to the lipid metabolism as it is essential for VLDL synthesis in the liver. Indeed, choline deficiency has been shown to cause liver damage. Other severe health outcomes associated with choline deficiency are muscle damage, aberrant gene expression, birth defects, decreased cognitive function, carcinogenesis, and cardiovascular disease. Choline is abundant in animal food sources, such as eggs, milk, meat, poultry, and fish, in addition to plant based sources including leafy vegetables, wholegrain products and legumes. So far, only the National Academy of Medicine (NAM) and the European Food Safety Authority (EFSA) have established Adequate Intakes (AI) for choline (respectively 425 mg/d for women and 550 mg/d for men or 400 mg/d for adults). For now there are no nationally representative estimates on choline intake in Norway and recommended dietary intake of choline for the Nordic countries has yet not been established.

Aim and hypothesis: To investigate the intake of total choline and individual choline forms in a healthy Norwegian population and map the food items contributing to the intake. Additionally, associations between choline intake and plasma concentrations of eleven metabolites involved in the one-carbon metabolism and serum lipids will be assessed.

Materials and methods: Data from the Hordaland Health study ´97-´99 was used for analyzing the intake of choline in a Norwegian population. Participants were born in 1925-27 or 1950-51 and were settled in Hordaland county (now part of Vestland). Baseline characteristics were retrieved through self-administered questionnaires and a non-fasting blood sample was taken at attendance. Dietary data was obtained using a semi-quantitative 169-item food frequency questionnaire (FFQ) designed according to a Norwegian habitual meal pattern. The intake of total choline and choline forms was analyzed from the FFQ using the USDA Database for the Choline Content of Common Foods 2008. Choline intakes were

energy-adjusted using the residual method and reported as means with standard deviations. Dietary intakes were energy-adjusted using the nutrient density method and were reported as g/1000 kcal or in energy percentage. To explore the relationship between choline intake and circulating levels of one-carbon metabolites and lipid metabolites, choline intake was modeled as a polynomial spline in a model with sex as an interaction term and adjusted for age, BMI and smoking.

Results: After exclusion, 5746 participants (43.6% men) were considered eligible for analysis. Nearly half of them (48.5%) were born in 1925-27, while the rest were born in 1950-51. The mean (SD) total choline intake was 265.2 (±55.9) mg/d in the total study population, and intakes were similar between men and women after energy-adjustment. The major food groups contributing to the intake of total choline were dairy, eggs, vegetables, grain products, and meat. Nearly half of the choline intake (43.5%) came from the lipid-soluble form PtdCho, which was mainly retrieved trough the intake of eggs, meat, grain products and vegetables.

The intake of choline was inversely associated with plasma concentrations of homocysteine (Hcy), glycine, and serine, and positively associated with methionine, cystathionine, cysteine, trimethyllysine, trimethylamine N-oxide (TMAO), and dimethylglycine, as well as plasma choline in men. In both men and women, a higher choline intake was associated with increased concentrations of total cholesterol (TC), LDL-cholesterol and triglycerides (TG).

Conclusion: To our knowledge, this study is the first to assess the intake of total choline and individual choline forms in a healthy Norwegian population. Compared to the AIs set by NAM and EFSA, most participants did not achieve the recommended intakes, even prior to energy-adjustment. Our findings suggest that choline intake affects the concentration of most metabolites involved in one-carbon metabolism, and has a Hcy-lowering effect, but increases the concentration of TML and TMAO in healthy men and women. A high intake of choline could also negatively affect lipid-profile, by increasing the serum concentrations of TC, LDL-cholesterol, and TG.

Sammendrag

Introduksjon: Kolin er et essensielt næringsstoff for mennesker til tross for at små mengder kan syntetiseres i leveren. Kolin er en viktig bestanddel av fosfolipider i cellemembraner, en sentral del av nevrotransmitteren acetylkolin, og er som forløper for metyldonoren betain også involvert i en-karbonmetabolismen. I mat og i kroppen finner vi kolin i de vannløselige formene fritt kolin, fosfokolin og glyserofosfokolin, og i de fettløselige formene fosfatidylkolin og spingomyelin. Fosfatidylkolin er en essensiell del av VLDL-partikler, og kolinmangel har vist å kunne føre til leverskade og fettlever. Andre alvorlige utfall som kan oppstå som følge av kolinmangel er skade på muskelceller, endret genregulering, fødselsskader, nedsatt kognitiv funksjon, og økt risiko for kreft og hjerte- og karsykdom. Vi finner mest kolin i animalske matvarer, som egg, melk, kjøtt og fisk, men også i plantebaserte kilder som bladgrønnsaker, fullkorn og belgvekster. Per dags dato er det fastsatt anbefalte inntak av kolin i form av Adekvate Inntak (AI) i Amerika og i Europa (henholdsvis 425 mg/d for kvinner og 550 mg/d for menn eller 400 mg/d for voksne). Det har enda ikke blitt estimert et nasjonalt representativt kolininntak i Norge eller blitt publisert anbefalt nivå for daglig inntak for nordisk populasjon.

Mål og hypotese: Estimere inntaket av totalt kolin og individuelle kolinformer i en frisk norsk befolkning, og undersøke hvilke matvarer som bidrar til dette inntaket. I tillegg vil assosiasjoner mellom kolininntak og blodkonsentrasjon av elleve en-karbonmetabolitter og serum lipider bli undersøkt.

Materiale og metode: Data fra Helseundersøkelsene i Hordaland ´97-´99 ble brukt for å undersøke inntaket av kolin i en frisk norsk befolkning. Deltakerne var født i 1925-27 eller 1950-51 og bosatt i Hordaland (nå del av Vestland). Informasjon om livsstil og helsevaner ble innhentet via selv-utfylte spørreskjemaer, og ved personlig frammøte ble det tatt en ikke-fastende blodprøve og målt høyde og vekt. Deltakerne fikk utdelt et matfrekvensskjema med 169 matvarer tilpasset et typisk norsk kostmønster som de fylte ut hjemme. Inntak av totalt kolin og individuelle kolinformer ble analysert ved bruk av beregninger av kolininnhold i matvarer publisert i USDA Database for the Choline Content of Common Foods 2008. Kolininntak ble energijustert ved bruk av residualmetoden, og inntaket er oppgitt i gjennomsnitt med standardavvik. Andre næringsinntak ble også energijustert og er oppgitt i g/1000 kcal eller i energiprosent. Assosiasjoner mellom kolininntak og metabolitter i en-

karbonmetabolismen og lipidmetabolismen ble undersøkt i en modell der det er tatt hensyn til kjønn som interaksjon, og justert for alder, BMI og røyking.

Resultat: Etter eksklusjon gjensto 5746 deltakere (43.6% menn) som ble inkludert i analysen. Omtrent halvparten (48.5%) var født i 1925-27, mens resten var født mellom 1950-51. Gjennomsnittlig (SD) totalt kolininntak var beregnet til 265.2 (±55.9) mg/dag, og inntaket hos menn og kvinner var omtrent likt etter energijustering. Matvarene som bidro mest til inntaket av kolin var meieriprodukter, egg, grønnsaker, fullkornsprodukter og kjøtt. Omtrent halvparten av inntaket var i form av den fettløselige kolinformen fosfatidylkolin (43.5%), som deltakerne hovedsakelig inntok fra egg, kjøtt, fullkorn og grønnsaker.

Inntak av kolin var negativt assosiert med plasma konsentrasjon av homocystein (Hcy), glysin, og serin, og positivt assosiert med metionin, cystationin, cystein, trimetyllysin, trimetylamin-N-oksid (TMAO), og dimetyl-glysin, samt med plasma kolin hos menn. For både menn og kvinner var et høyere kolininntak assosiert med økt serumkonsentrasjon av totalkolesterol, LDL-kolesterol og triglyserider.

Konklusjon: Dette er til vår kunnskap den første studien som undersøker inntaket av totalt kolin og individuelle kolinformer i en frisk norsk populasjon. Sammenlignet med anbefalte AI satt for amerikansk og europeisk populasjon, hadde de fleste deltagerne et inntak under anbefalingene, selv før energijustering. Våre funn tyder på at kolininntak påvirker konsentrasjoner av de fleste en-karbonmetabolitter og kan bidra til redusert nivå av Hcy i plasma, men også til økt konsentrasjon av TML og TMAO. Et høyt kolininntak kan videre ha en negativ effekt på lipidprofil, ved å bidra til økt serum totalkolesterol, LDL-kolesterol, og triglyserider.

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List of abbreviations

PtdCho	Phosphatidylcholine
ACh	Acetylcholine
FC	Free choline
PC	Phosphocholine
GC	Glycerophosphocholine
SM	Sphingomyelin
Lyso-PtdCho	Lysophosphatidylcholine
VLDL	Very-low-density lipoprotein
CDP-choline	Cytidine diphosphocholine
PE	Phosphatidylethanolamine
PEMT	Phosphatidylethanolamine-N-methyltransferase
EK	Ethanolamine kinase
ECT	Phosphoethanolamine cytidyltransferase
EPT	Ethanolaminephosphotransferase
CK	Choline kinase
CT	CTP:phosphocholine cytidyltransferase
CPT	Choline phosphotransferase
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
Hcy	Homocysteine
CHDH	Choline dehydrogenase
BADH	Betaine aldehyde dehydrogenase
MS	Methionine synthase
mTHF	Methyltetrahydrofolate
BHMT	Betaine-homocysteine S-methyltransferase
DMG	Dimethylglycine
THF	Tetrahydrofolate
ChAT	Choline acetyltransferase
TMAO	Trimethylamine-N-oxide
TMA	Trimethylamine
FMO	Flavin-containing monooxygenase
TML	Trimethyllysine

NAFLD	Non-alcoholic fatty liver disease
TG	Triglyceride
NTD	Neural tube defect
CVD	Cardiovascular disease
NAM	National Academy of Medicine
UL	Tolerable upper intake level
EAR	Estimated Average Requirement
AI	Adequate Intake
EFSA	European Food Safety Authority
RDA	Recommended Dietary Allowance
USDA	United States Department of Agriculture
HUSK2	The Hordaland Health Study 1997-99
FFQ	Food frequency questionnaire
TC	Total cholesterol
HDL	High-density-lipoprotein
LDL	Low-density-lipoprotein
BMI	Body mass index
E%	Energy percentage
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid

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1. Introduction

1.1. Choline

Choline has been officially recognized as an essential micronutrient for humans since 1998. Even though humans can synthesize small amounts of choline endogenously in the liver, dietary consumption of choline is necessary for optimal health and to maintain several biological functions. In fact, it is observed that cells grown in culture have an absolute requirement of choline, and when deprived of choline they die of apoptosis (1). Choline is involved in liver, muscle and brain functions, and plays a role in diverse processes such as cellular signaling, hepatic lipid metabolism, methylation-dependent biosynthesis of molecules, including epigenetic regulation and gene expression (2, 3). Most choline in our body is found as phosphorylated choline compounds that are elementary structural phospholipids in most cell membranes. In fact, phosphatidylcholine (PtdCho) is the predominant phospholipid in mammalian cell membranes (4). Choline is also linked to one-carbon metabolism through irreversible conversion to betaine, an important methyl group donor. Last, but not least, choline is a precursor for the neurotransmitter acetylcholine (ACh), which is essential for brain functions such as memory, learning and sleep (2, 5).

1.2. Digestion, absorption and transport

Choline can be found as free choline (FC) or in esterified forms, including phosphocholine (PC), glycerophosphocholine (GC), PtdCho, and sphingomyelin (SM). FC, GC and PC are water-soluble compounds, while PtdCho and SM are lipid-soluble. Chemical structures of the choline forms are depicted in **Figure 1**.

The majority of lipid-soluble choline is found as structural parts of cell membranes in both foods and in our body (2). Out of the five choline compounds, PtdCho is the most consumed form, while SM is rarely to be found in foods. The concentration of PtdCho is high in foods of animal origin, such as eggs, meat, liver, fish, chicken, and dairy. Overall, total choline content per unit of weight is well documented to be higher in animal food sources than in plant-based sources (6). Most plant-based foods contain more water-soluble choline forms, and we find FC, PC and GC mostly in foods like fresh vegetables, potatoes, grain products and soybeans, in addition to fish and milk, including human milk (6). Most choline consumed during infancy is therefore water-soluble, while later on we get the most of our dietary choline from lipid-soluble forms, mainly PtdCho.

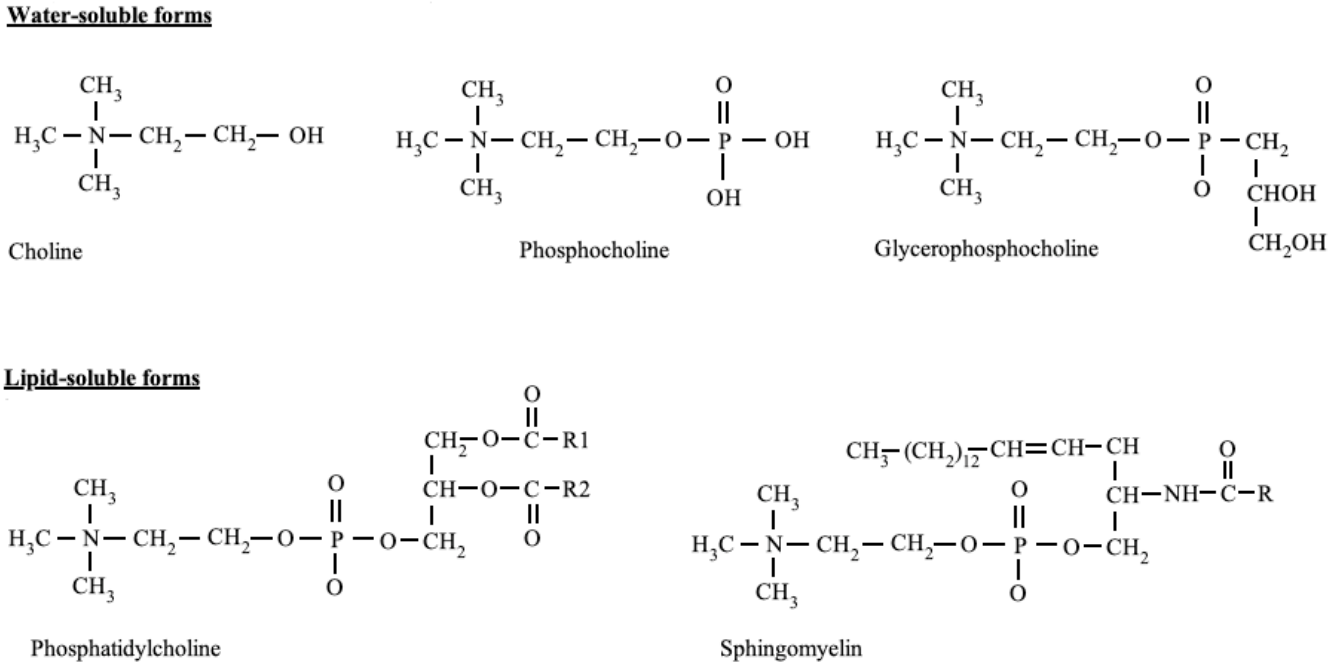

Figure 1. Chemical structures of choline forms. Choline, trimethyl-beta-hydroxy-ethylammonium. Water-soluble; free choline, phosphocholine and glycerophosphocholine. Lipid-soluble; phosphatidylcholine and sphingomyelin. R represents a fatty acid chain.

Digestion, absorption and metabolism of the choline compounds differ, particularly between the lipid-soluble and water-soluble forms. The intestinal digestion is mediated by both pancreatic and mucosal enzymes and begins in the proximal small intestines (7). FC is extracted from the water-soluble choline forms GC and PC by pancreatic enzymes, before it is absorbed in the small intestines by mediated transport (4). Prior to absorption, SM is degraded to sphingosine and FC by mucosal brush-border enzymes, while PtdCho is partly hydrolyzed to lysophosphatidylcholine (lyso-PtdCho) and free fatty acids by pancreatic phospholipases (7). Some lyso-PtdCho is further hydrolyzed to GC, and ultimately to FC. FC and lyso-PtdCho are then absorbed to the enterocytes, where lyso-PtdCho is packed in chylomicrons and enters the lymphatic circulation. Via the lymphatic circulation, lipid-soluble choline is delivered to muscles, adipose tissue and other tissues before reaching the liver. FC in the enterocytes can be converted to PtdCho again, which enters the lymphatic circulation as well, but most FC is transferred to the portal vein and transported directly to the liver (7).

The liver is an important organ for metabolism and storage of choline, and choline is absorbed from the portal circulation at high rates. In fact, the liver is dependent of dietary choline, as

dietary choline deficiency in humans causes hepatic fat accumulation and liver dysfunction (8). In the liver, absorbed choline is partly used for synthesis of PtdCho, while some is oxidized to betaine (7). Most of the newly synthesized PtdCho is secreted into bile, before 95% of it is reabsorbed in the intestines and returned to the liver and other organs. The amount secreted in bile actually exceeds most people´s dietary PtdCho intake, and an efficient absorption of choline is therefore important to maintain choline homeostasis (4, 7).

Even though most choline is metabolized and stored in the liver, all tissues require choline, including the brain, kidneys, lungs and muscles (1). The brain has a specific carrier mechanism that transports FC across the blood-brain barrier at a rate proportional to serum choline concentrations (2). The choline transporter in neonate brains has an especially high capacity due to increased demands during cellular development. In the brain, choline is stored as PtdCho in cell membranes and subsequently converted to ACh when it is needed for neuronal activity (8). Most choline that is taken up in the kidneys is oxidized to betaine which there serves as an osmotic regulator, while in the lungs, PtdCho is an essential component of the pulmonary surfactant (1, 9). Choline deficiency is also associated with damage to the skeletal muscles detected by leakage of creatine phosphokinase from muscle cells caused by decreased cell integrity (10). The dysfunction to the organ is not observed to be permanent and resolves when choline is added to the diet again.

1.3. Main fates of choline

1.3.1. Phosphatidylcholine and de novo synthesis

The main metabolic fate of absorbed dietary choline is phosphorylation to PtdCho in the liver (4). PtdCho is the most abundant form of choline in the body, and accounts for about 95% of the total choline pool in humans (4). It is an essential phospholipid in most cell membranes and has a particularly important role in liver metabolism. In the liver, PtdCho is required for solubilization of bile salts for secretion, and for packaging and export of triglycerides, as it is a structural part of very-low-density lipoproteins (VLDL) (3, 4).

Choline is converted to PtdCho in a two-step reaction referred to as the cytidine diphosphocholine (CDP-choline) pathway, which is one of two branches of the Kennedy pathway (**Figure 2**) (9). The other branch of the Kennedy pathway is synthesis of the phospholipid phosphatidylethanolamine (PE), which subsequently can be used for synthesis of PtdCho. In the CDP-choline pathway, choline is first phosphorylated to cytidine

diphosphocholine, which is why the pathway is called the CDP-choline pathway, and then to PtdCho. The CDP-choline pathway occurs in the nucleus of all nucleated cells, but in the liver, it only accounts for about 70% of the PtdCho synthesized (6). The remaining 30% of hepatically produced PtdCho originates from conversion of PE, an alternative pathway that does not require choline but ethanolamine from dietary phospholipids and diacylglycerol. PE from the Kennedy pathway can be converted to PtdCho in a reaction catalyzed by the enzyme phosphatidylethanolamine-N-methyltransferase (PEMT), requiring three molecules of ATP and methionine, respectively. The pathway is referred to as the PEMT-pathway, and this pathway is also the major and only known pathway for *de novo* choline synthesis in humans (4). By various phospholipases, choline can be endogenously produced from PtdCho formed from the PEMT-pathway. This synthesis mainly occurs in the liver but can occur in other tissues as well. It can be a significant source of choline next to dietary intake, but evidence has revealed that it is insufficient to cover our biological needs and choline is therefore an essential nutrient for humans (1).

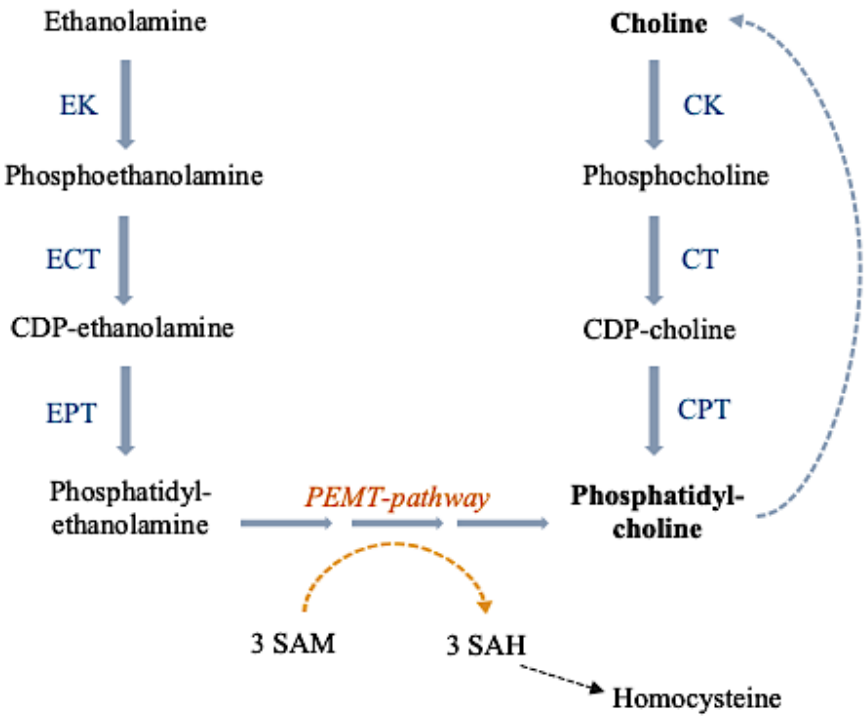

Figure 2. Metabolism of PtdCho via the Kennedy pathway and the PEMT-pathway. EK, ethanolamine kinase; ECT, phosphoethanolamine cytidyltransferase; EPT, ethanolamine-phosphotransferase; CK, choline kinase; CT, CTP:phosphocholine cytidyltransferase; CPT, choline phosphotransferase; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

The ability to synthesize choline endogenously is limited by the production and capacity of the PEMT-enzyme. Expression of the PEMT-gene is modulated by genetics, hormonal status, and sex. The promotor region of the gene has several estrogen responsive elements, and thus the gene expression is higher in most premenopausal women compared to men and postmenopausal women not treated with estrogen (11). Other factors that can influence an individual´s requirement for choline will be further discussed in chapter *1.5.4. Individual variations in choline requirements*.

1.3.2. Betaine and one-carbon metabolism

Choline is irreversibly oxidized to betaine in the liver and the kidneys in a two-step reaction catalyzed by choline dehydrogenase (CHDH) and betaine aldehyde dehydrogenase (BADH) (6). Betaine is an important osmolyte in the kidneys, while in the liver it functions as a methyl group donor in the one-carbon metabolism. Through its metabolite betaine, choline is closely interrelated with the metabolism of folate and methionine (**Figure 3**), and the availability of these metabolites influences the dietary requirements of one another (1, 2).

One-carbon metabolism is a set of biochemical enzymatic reactions where one-carbon groups, such as methyl and formyl, are transferred between compounds. It consists of several intertwined pathways and cycles, many of them dependent of B-vitamins as co-factors and co-substrates. All cells require one-carbon units for diverse processes such as nucleotide synthesis, DNA-methylation and epigenetic maintenance, reductive metabolism, redox defense, and amino acid homeostasis (12). Disturbances in metabolites involved in one-carbon metabolism have been linked to the development of several chronic diseases (13).

Three of the reaction-pathways in the one-carbon metabolism involve reduction of homocysteine (Hcy) levels, which is formed from S-adenosylhomocysteine (SAH) originating from demethylation of methionine to S-adenosylmethionine (SAM) (**Figure 2**) (14). SAM is an universal methyl group donor that for instance donates three methyl groups for the conversion of PE to PtdCho catalyzed by the PEMT-enzyme (5). In fact, it is estimated that about 50% of all Hcy produced originates from SAH formed in the PEMT-pathway (6).

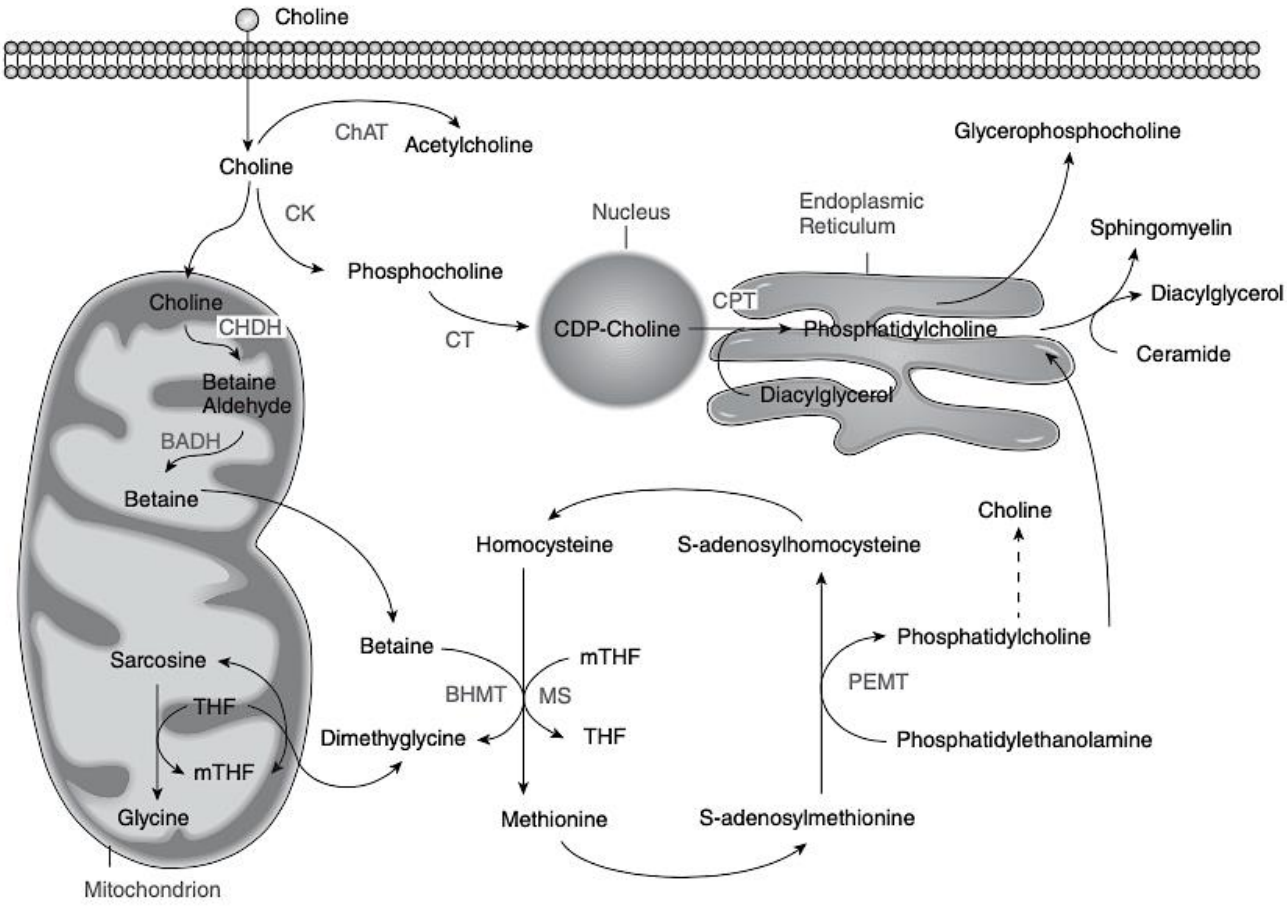

Figure 3. Choline is involved in one-carbon metabolism through its metabolite betaine and interrelated with the pathways of folate and methionine. ChAT, choline acetyltransferase; CK, choline kinase; CT, CTP:phosphocholine cytidyltransferase; CPT, choline phosphotransferase; CHDH, choline dehydrogenase; BADH, betaine aldehyde dehydrogenase; BHMT, betaine-homocysteine S-methyltransferase; MS, methionine synthase; mTHF, methyltetrahydrofolate; THF, tetrahydrofolate; PEMT, phosphatidylethanolamine-N-methyltransferase. Figure from Zeisel et. al. (2).

The first Hcy-reducing pathway is the transsulfuration pathway. Hcy is converted to cystathionine before irreversible conversion to cysteine. However, methionine is an essential amino acid, and in case of a methionine imbalance Hcy is remethylated to methionine instead, via two methionine conserving pathways (14). In most tissues, the remethylation is catalyzed by methionine synthase (MS), which uses methyltetrahydrofolate (mTHF) as a substrate, and vitamin B12 as cofactor. This pathway is dependent on folate as a methyl group donor and is part of the folate cycle in one-carbon metabolism. The other pathway occurs predominantly in the liver, where about half of the remethylation of Hcy is catalyzed by betaine-homocysteine S-methyltransferase (BHMT). In this reaction, betaine is used as a methyl group donor,

linking choline closely to the one-carbon metabolism. When donating a methyl group, betaine is converted to dimethylglycine (DMG) (6). DMG is further degraded to sarcosine, and ultimately glycine, a reaction that provides new methyl groups for remethylation of tetrahydrofolate (THF) to mTHF, which again can be used as a substrate in the folate-dependent remethylation of Hcy (**Figure 3**) (15).

The two pathways where Hcy is remethylated to methionine are interchangeable and depend on the availability of the potential methyl group donors, folate and betaine. Disturbances in one of these pathways will cause compensatory changes in the other to maintain the rate of remethylation (2). It is usually believed that in case of enzymatic defects or micronutrient deficiency, Hcy can accumulate and plasma levels of Hcy increase (14).

1.3.3. Acetylcholine

In the nervous system, choline is needed as a precursor for ACh (4). ACh operates both in the central and peripheral nervous system and is involved in various neuronal functions, such as memory, learning, mood, sleep, and muscle control. It is synthesized from choline and acetyl coenzyme A by the enzyme choline acetyltransferase in cytosol of the cells (6). That choline was part of this essential neurotransmitter was discovered in the early 20th century, and in the 1970s it was observed that dietary choline intake directly modulates the synthesis and release of ACh (16). This discovery led to the beginning of considering whether choline could be used as a therapeutic agent for neurological disorders. All cholinergic neurons require choline for the synthesis of ACh, and choline transporters in neurons have a particularly high affinity for choline. In the neurons, choline is stored as part of phospholipids in the cell membrane, and is a large precursor pool for synthesis of ACh when needed (2).

1.3.4. TMAO

Dietary choline and betaine intake can also affect the plasma concentration of trimethylamine-N-oxide (TMAO), a metabolite that has been linked to several metabolic diseases, such as cardiovascular disease and diabetes (17). Choline, betaine and carnitine are all dietary precursors of TMAO. Choline and choline esters that are not absorbed in the small intestine continue to the large intestine where gut bacteria use FC and PtdCho as an energy substrate and carbon source to metabolize trimethylamine (TMA) (7). TMA is absorbed and transported to the liver where it is oxidized to TMAO by flavin-containing monooxygenases (FMOs) (6). TMA and TMAO can additionally be found in fish or converted from the

precursor trimethyllysine (TML) which is abundant in most animal- and plant-protein (18). The plasma level of TMAO is primarily determined by three factors, which are 1) the intake of dietary precursors like choline, betaine and carnitine, 2) bacterial formation of TMA in the large intestine, and 3) oxidation of TMA to TMAO by FMOs in the liver (19). It is therefore interesting to address the associations between dietary choline and TMAO levels, which will be further discussed in chapter *1.4.6. Cardiovascular disease.*

1.4. Health implications

1.4.1. Liver damage and NAFLD

Choline deficiency is associated with liver damage and non-alcoholic fatty liver disease (NAFLD), a state caused by fat accumulation in the liver, followed by inflammation, fibrosis and cirrhosis (3). NAFLD affects about 10-35% of the general population and is a multifactorial disease, influenced by e.g. diet, exercise and genetics. The primary etiology is long-term caloric excess and/or overconsumption of alcohol (20). However, newer research points to several mechanisms behind the development of NAFLD being associated with choline availability and other metabolites involved in one-carbon metabolism. Clinical studies have shown that humans fed with total parenteral nutrition-solutions devoid of choline develop signs of liver damage, and eventually fatty liver disease (21). This was also observed in men fed defined diets low in choline (22). When a source of choline is added to the diet again, the damage to the liver resolves.

The mechanism behind is an impairment in the synthesis of VLDL-particles, which requires PtdCho. Triglycerides (TG) are exported from the liver packed in VLDL-particles, and an alteration in the VLDL synthesis will alter the hepatic lipid metabolism, and eventually cause fat accumulation in the liver (3, 4). Interestingly, it is observed that some premenopausal women do not develop fatty liver or liver damage on a choline deficient diet, when most men and postmenopausal women do (10, 23). As mentioned earlier, this is because production of the PEMT enzyme, that is responsible for synthesis of PtdCho and *de novo* synthesis of choline, is induced by estrogens. Moreover, mice models that lack the PEMT enzyme develop steatohepatitis and die of liver failure after few days when fed a choline deficient diet (24). This suggests that during choline deficiency or increased demands of choline, e.g. starvation, pregnancy or lactation, the PEMT pathway is essential for maintaining PtdCho and choline concentrations, and for preventing fat accumulation and damage to the liver (4).

1.4.2. Gene expression and methyl metabolism

Epigenetic alterations of DNA regulate whether a gene is expressed or not, without changing the DNA sequence permanently, for instance through DNA methylation (2). Epigenetic alterations are responsive to environmental lifestyle factors, including the intake of dietary sources of methyl group donors, such as folate, choline, betaine and methionine (25). These micronutrients have various, but central roles in the formation of the universal methyl group donor SAM. SAM can donate its methyl group in more than 80 biological methylation reactions, including the methylation of PE to PtdCho through the PEMT-pathway as earlier described, but also in methylation of DNA, RNA, and proteins (26). Low availability in choline may negatively affect the SAM pool, which subsequently alters DNA methylation. For instance, it has been observed that choline deficiency during pregnancy alters DNA methylation and gene expression in several tissues, including brain and liver, which causes permanent changes to the developing fetus (2).

1.4.3. Neurological development and brain function

Adequate fetal and perinatal nutrition is essential for development and function of organs, including the brain. Micronutrients such as iron, zinc and folate, are recognized to alter brain development. In addition to these familiar micronutrients, evidence suggests that choline is essential for optimal brain formation and function as well (27). The human milk is rich in water-soluble choline, and neonates have a much higher plasma concentration of choline compared to adults (28). PtdCho and SM are essential phospholipids in all cell membranes, and the rapidly developing tissues in neonates require large amounts of choline for membrane synthesis (29). As mentioned earlier, choline is needed in the synthesis of the neurotransmitter ACh, and after oxidation to betaine it is an important contributor to DNA methylation which alters gene expression (6, 27). During gestation and the perinatal period, choline availability is critical for the cholinergic organization of the brain and can impact brain functions throughout the lifespan (29). In rodents, it is observed that maternal choline intake during the perinatal period alters the anatomical and biochemical development, as well as affecting the cognitive function, such as learning and memory in later life (27). Studies on choline intake and its impact on the central nervous system in humans are few. Still, choline is observed to have beneficial effects on neurological function in small trials, and choline supplementation is suggested as a useful treatment for several neurological disorders (2).

1.4.4. Birth defects

Not only does choline impact brain development in fetus, but a high choline concentration in the spinal cord is also important for neural tube closure (27). Neural tube defects (NTDs) and other birth defects could possibly be prevented by identifying dietary components that influence its etiology. For more than 30 years, folate supplementation during the periconceptional period has been recognized to lower the risk of NTDs, and supplementation is therefore recommended before and during gestation (25). Like folate, choline has also been associated with NTDs and other birth defects, such as cleft lip, defect in male urethra, heart defects and congenital diaphragmatic hernia (2). Evidence show that the availability of several methyl donors including choline and methylated folate, alters DNA-methylation, which may be one of the mechanisms behind the development of NTDs (25). A large population-based study of periconceptional dietary choline intake in Californian women supports that choline, and possibly betaine, is involved in the complex etiologies of NTDs, irrespective of folate intake (25). They observed that women with a choline, betaine, and methionine intake above the 75th percentile had a 4-fold lower risk of NTD-affected pregnancies compared to women with an intake below the 25th percentile. In addition to this, a choline intake above 290 mg/d was associated with decreased risk of NTDs.

1.4.5. Carcinogenesis

Choline deficiency has been associated with carcinogenesis and tumor progression in both animals and humans, but the mechanisms behind are not fully understood. Studies of choline deficiency in rats are the first published studies where a micronutrient deficiency caused development of cancer, without any carcinogen administered (2). The first studies were published in the 1950s, but the discovery was not recognized or confirmed before 30 years after (30, 31). Rats with chronic choline deficiency developed spontaneous hepatocarcinoma and showed increased sensitivity to administered carcinogens. This suggests that choline deficiency has both cancer-initiating and cancer-promoting effects (31). The liver was the most frequently occurring site of neoplasms, originating from alterations in liver structure such as fatty liver, fibrosis and cirrhosis, which ultimately caused hepatocellular carcinoma in some animals. In humans, choline deficiency in women has been associated with prevalence of breast cancer in a large population-based study (26). The women in the highest quantile of choline consumption had a 24% reduced risk of breast cancer, compared to women in the lowest quantile. On the other hand, it has also been established that both breast-, prostate- and brain cancer cells contain elevated levels of phosphocholine and total choline compared to

healthy tissue (32). Although animal studies imply a causal relationship between choline deficiency and carcinogenesis, more research is needed before any conclusions of its role in human carcinosis can be drawn.

1.4.6. Cardiovascular disease

Cardiovascular diseases (CVD) are multifactorial diseases influenced by genetic and environmental factors. To investigate the single effect of one factor can therefore be challenging, and evidence of the associations between choline intake and CVD is contradicting. It has been observed that a high plasma choline level is associated with risk of chronic diseases, including CVD (5). However, long-term dietary choline intake does not necessarily correlate with plasma choline levels, and the impact of dietary choline intake on development of CVD is unclear.

The reported associations between dietary intake of choline and CVD have been related to its effect on concentrations of Hcy and TMAO, and the pathways involved could possibly either increase or decrease the risk of CVD (6). Hcy is an independent risk factor for atherosclerosis (14), and hyperhomocysteinemia (elevated Hcy levels) is associated with various health issues, including CVD, ischemic stroke, neurological disorders and diabetes (33). The mechanisms behind the association involves damage to the endothelium and smooth muscle cells, decreased flexibility of blood vessels, increased inflammation and increased blood pressure (34). Severely high Hcy levels (>100 μmol/L) are caused by rare genetic mutations, while a moderate elevation (>15 μmol/L) is more common, and can be related to genetics or environmental factors such as age, diet, smoking, caffeine and alcohol consumption (34). Regarding dietary factors, deficiency in micronutrients involved in the remethylation of Hcy to methionine, including folate, vitamin B6, vitamin B12, betaine and its precursor choline, can cause increased Hcy levels (1, 34). Under conditions of an impaired folate-dependent remethylation of Hcy e.g. during folate deficiency, the betaine dependent pathway may compensate to prevent accumulation of Hcy. It is observed that high doses of dietary choline and betaine together are inversely associated with Hcy, and so is a high intake of the water-soluble choline forms GC and PC (35). PtdCho supplementation can lower fasting Hcy levels after a methionine load (36). Because of its Hcy-lowering effects, betaine has for a long time been used in the treatment of patients with hyperhomocysteinemia caused by genetic mutations, but evidence suggest that the intake of its precursor choline could be a potential treatment as well (1, 37).

Like Hcy, elevated plasma levels of TMAO have been positively associated with progression and risk of atherosclerosis and CVD (6). The adverse effects of choline deficiency are discussed earlier, but since dietary choline intake is positively correlated with TMAO levels, this might be a cause of concern regarding cardiovascular health. Extremely high TMAO levels can cause fishy body odor, which have been observed after large oral doses of choline (2). The associations between TMAO and risk of CVD have recently gained increasing interest, and current studies suggest that a high circulating level could be a potential risk factor for CVD and mortality (38). The proposed mechanisms behind involve accelerated formation of atherosclerotic plaque caused by endothelial dysfunction, inflammation, altered lipid homeostasis and promotion of oxidative stress, but the complete underlying mechanisms are still undefined (18, 38). Though higher TMAO levels have been observed in patients with CVD, it is also uncertain whether TMAO is a mediator of disease, or rather a prognostic biomarker.

A diet restricted in foods containing choline and other dietary precursors of TMAO, like carnitine and betaine, has been suggested as a potential target point to prevent increased TMAO levels. Choline is found in a great variety of food sources. Some have been associated with increased risk of CVD, such as eggs and red meat, while foods like green vegetables, whole grain and fish that also contain choline compounds, are known to have a beneficial impact on cardiovascular health. However, the food item that causes the highest increase in TMAO after consumption is fish, which contains preformed TMAO in addition to the precursor choline (39). The TMAO response to choline intake can be variable since the intestinal formation of TMA from choline is also highly dependent of gut microbiota (18). Moreover, a systematic review of six studies found no associations between dietary intake of TMAO precursors (choline and betaine) and incidence of CVD (19). Due to contradicting evidence and considering the amount of foods containing choline compounds, a diet restricted in TMAO-generating nutrients is not necessarily recommended and should be implemented carefully (19).

1.4.7. Toxicity and tolerable upper intake level

Not only is dietary deficiency of choline associated with several adverse health outcomes, but so are high doses of choline. Humans administered high doses of choline experience toxic effects, including hypotension, fishy body odor and cholinergic side effects such as sweating and diarrhea (1). Hypotension was first observed in a study in seven patients treated with high

doses of oral choline to reduce symptoms of Alzheimer´s disease (40). When the patients received 7.5 g/d of choline, some experienced nausea, diarrhea, and a small decrease in blood pressure. These effects were not observed after a dose of 4.0 g/d, and thus 7.5 g/d was the lowest-observed-adverse-effect-level. With additional results from four other studies where oral choline administration caused cholinergic side effects and fishy body odor, the National Academy of Medicine (NAM) could estimate a tolerable upper intake level (UL) of 3.5 g/d for adults in 1998 (1). Derived from the adult UL, an UL for adolescents was set to 3 g/d (14-18 years) and 2g/d (9-13 years), and 1 g/d for children (1-8 years).

1.5. Dietary intakes and recommendations

1.5.1. US recommendations

In 1998, NAM officially recognized choline as an essential nutrient for humans after the discovery that the endogenous synthesis was insufficient to cover the biological needs (1). This was first observed in a study in healthy men, where low intakes of choline caused liver dysfunction shown by elevated alanine aminotransferase levels (22). NAM then published the first recommendations for choline intake for the American population, based on this single study in choline depletion. The amount needed to prevent liver dysfunction was thus the primary criteria for the established recommendations. Due to limited scientific evidence, it was not possible to calculate an Estimated Average Requirement (EAR), leading to only recommended Adequate Intakes (AIs) of total choline which are provided in **Table 1** (1).

In the choline depletion study, it was observed that choline supplementation of about 7 mg/kg/day prevented liver damage. Based on this, the AI of choline for adults was set to 550 mg/d for men and 425 mg/d for women. During pregnancy, large amounts of choline are transported to the developing fetus through placenta, depleting the mother of choline (5). Pregnant women therefore have an additional requirement, and the AI was set to 450 mg/d during pregnancy. A substantial amount of choline is also secreted in the human milk when lactating, which contains about 1.5-2 mmol/L of total choline (1). The AI for lactating women is therefore set to 550 mg/d, even higher than during pregnancy. For children and adolescents, AIs were extrapolated from adult requirements dependent on body mass and calculated to a range from 125-375 mg/d. For infants, mean consumption of human milk and its content of choline was used to estimate an AI of 125-150 mg/d.

1.5.2. European recommendations

In 2016, 18 years after the first recommendations were published in the US, the European Food Safety Authority (EFSA) published Dietary Reference Values for Choline for the European populations (**Table 1**) (41). Even with more data available to calculate the recommended intakes, they still agreed with NAM that an EAR for choline could not be derived, and EFSA recommended AIs for the European population as well. The recommended AIs were based on individual food consumption data from twelve dietary surveys conducted in nine European countries between 2000-2011 (41). The mean intake of choline observed in healthy European populations was about 370 mg/d. In addition, they reviewed 11 choline depletion/repletion studies, whereas the result from one was taken in consideration when estimating the AIs. This study reported that an intake of 400 mg of choline per 70 kg bodyweight per day was the amount needed to improve symptoms of organ dysfunction caused by choline depletion in 70% of the subjects (10). Based on this and the mean intake measured in dietary surveys, the EFSA Panel set an AI of choline for all adults, both men and women, to 400 mg/d (**Table 1**). The AI for infants aged 7-11 months is 160 mg/d, based on extrapolation from estimated choline intake of exclusively breastfed infants from 0-6 months which was calculated to 120 mg/d. AIs for children and adolescents, 1-17 years, were extrapolated from the adult AI and range from 140-400 mg/d, based on growth factors. Due to the increased requirements during pregnancy and lactation, the AIs were estimated to 480 mg/d and 520 mg/d, respectively.

At this date, a Recommended Dietary Allowance (RDA) has yet not been established due to lack of data on choline intakes, and there is no consensus on dietary choline requirements. Recommended daily intakes of choline for the Nordic populations have not been estimated and remains a point of contention as well. However, an updated version of the Nordic Nutrition Recommendations will be launched in 2022 and will be the first NNR to include choline as an essential nutrient (42).

Table 1: Current recommended adequate intakes (mg/d) for American and European populations.

		NAM (1998)		EFSA (2016)
		Male	**Female**	**Male/Female**
Infants	0-6 months	125		120
	7-12 months	150		160
Children	1-14 years	200-375		140-340
Adolescents	15-18 years	550	400	400
Adults	>18 years	550	425	400
Pregnancy	-	-	450	480
Lactation	-	-	550	520

American recommendations set by NAM; National Academy of Medicine (1).
European recommendations set by EFSA; European Food Safety Authority (41).

1.5.3. Dietary choline intake in adults

The first database of choline composition in foods was released in 2004 by the US Department of Agriculture (USDA), and the first report on dietary choline intake was published in the US the year after (43, 44). To this date, total choline intake has been investigated in a range of countries worldwide, including the USA (45, 46), Canada (47), China (48), Mexico (49), Taiwan (50), New Zealand (51), and several European countries (52). Total choline intake from twelve national food surveys conducted in nine European countries (Finland, France, Germany, Ireland, Italy, Latvia, The Netherlands, Sweden and the UK) has been analyzed and compared by Vennemann et.al., which was the data behind the recommendations set by EFSA (52). Overall, the average choline intake in European subpopulations ranged from 291-468 mg/d in adults. The highest choline intakes have been measured in Sweden, with a mean intake of 468 mg/d in men and 374 mg/d in women (53). Even though there are some variations, the estimated dietary intake of choline has been below the recommendations from both NAM and EFSA in most population groups studied.

1.5.4. Individual variations in choline requirements

There are individual variations in dietary choline requirements, modulated by dietary composition, sex, hormonal status, and genetics (11). Humans can synthesize choline endogenously, and the amount synthesized can decrease an individual´s dietary requirement. As discussed earlier, the amount of endogenous synthesis depends on the capacity of the PEMT enzyme, which is estrogen-responsive. Therefore, premenopausal women with higher estrogen levels have an increased potential of *de novo* choline synthesis than men and postmenopausal women not treated with estrogen (5). Premenopausal women can be relatively resistant to choline deficiency, even under conditions that increase the requirement, like pregnancy and lactation. When deprived of choline, it is therefore observed that premenopausal women less commonly develop signs of organ dysfunction compared to men and postmenopausal women (10). However, this only accounts for about half of all premenopausal women, as many have a genetic polymorphism in the PEMT-gene that makes it unresponsive to estrogen (3). In addition, it is observed that epigenetic variations affecting the expression of other enzymes involved in choline metabolism, such as BHMT and CHDH, also affect the dietary requirement of choline and the susceptibility to develop organ dysfunction on a choline deficient diet (2, 3).

Another factor that can influence the dietary requirement of choline is the intake of other nutrients interrelated with the metabolism of choline, particularly folate, methionine and betaine. As mentioned earlier, methylated folate and betaine both serve as methyl group donors for remethylation of Hcy. During folate deficiency, choline requirements increase as betaine becomes the primary methyl group donor in the remethylation of Hcy (2). Conversely, dietary folate requirements increase during choline deprivation followed by low betaine availability. A dietary intake of betaine itself can also reduce the amount of choline that is oxidized to betaine, and thereby decrease the dietary choline requirements (2).

1.6. Dietary sources of choline

The USDA Database for the Choline Content of Common Foods was published in 2004 as the first database of choline content in foods (44). It included 434 food items common in the North American diet. An updated and expanded release was published in 2008 and the database now contains 634 food items (54). Foods were analyzed for all five forms of choline, FC, GC, PC, PtdCho and SM, and total choline was defined as the sum of them. The choline metabolite betaine was also included in this database. However, since betaine is generated from choline by two irreversible reactions and cannot be converted back to choline, it is not considered as part of the total choline intake and is therefore not considered in our intake assessment.

A great variety of foods contain significant amounts of choline and choline compounds, both animal and plant sources. **Figure 4** shows the seven food groups with the highest content of total choline per weight of unit. Ranked from highest to lowest in choline content are whole eggs, meat and fish, whole grains, breakfast cereal, some vegetables and fruits, milk, and fats and oils (54). Eggs are particularly high in choline, with a content of about 250 mg per 100 g.

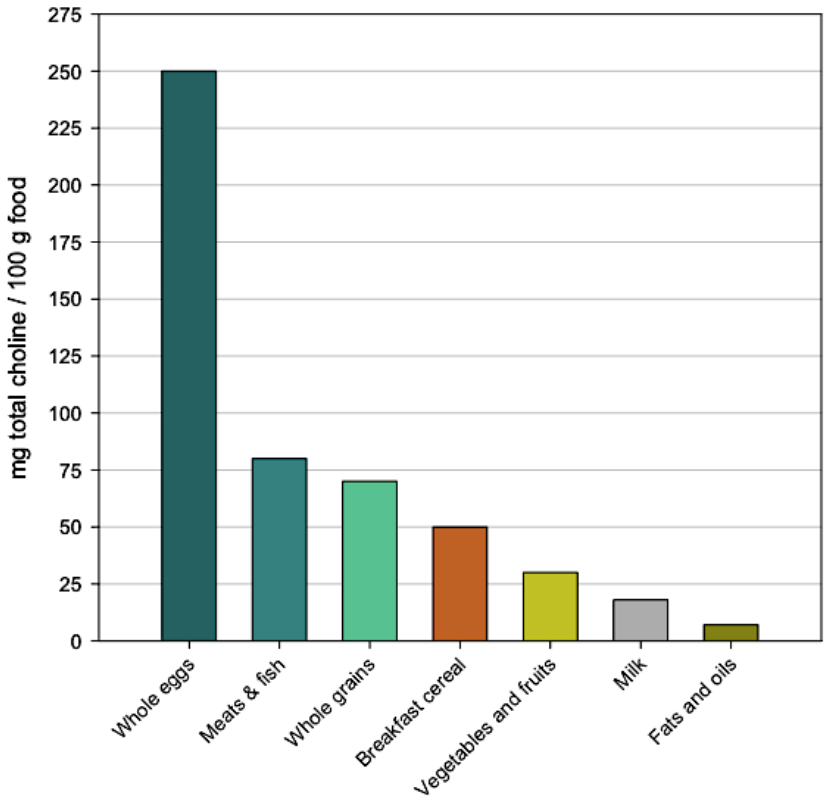

Figure 4. Relative content of total choline in seven food groups. Figure obtained from USDA Database for the Choline Content of Common Foods 2008 (54).

The content of water-soluble and lipid-soluble choline differs between food groups. In vegetables, fruits, grains and other plant-based food sources, we find most of the choline in the water-soluble forms FC, PC and GC. Lipid-soluble choline, particularly PtdCho, is abundant in animal foods such as eggs, milk, chicken, liver, beef, and fish. In both human tissue and in foods, PtdCho is much more abundant than SM, and the majority of the intake of lipid-soluble choline comes from PtdCho. Apart from animal food sources, PtdCho is also found in vegetable foods, including legumes and whole grains (55). In addition, many prepacked foods contain lecithin as an emulsifying agent, which is an industrially produced PtdCho-rich fraction (1).

Until this date, the USDA Database for the Choline Content of Common Foods is the only database on choline content in food items. The values of choline and choline compounds in foods are not included in most food composition tables, including the Norwegian one (56). All analyses of choline intake, also in a Norwegian population, must therefore be calculated using the database from the USDA which is based on common American food items and their choline content.

2. Aims and hypothesis

To establish appropriate dietary recommendations of choline for Nordic populations, there is a need for accurate estimates of the dietary intakes of choline in the countries concerned. Knowledge on food items contributing to the total choline intake and intake of the individual choline forms is limited, and so is data on the potential health effects associated with choline intake. It is important to investigate these areas to get more insight into the habitual choline intake in Norway and provide knowledge for dietary recommendations.

The primary aim of this thesis is to assess and estimate the average daily intake of total choline, water-soluble choline (FC, PC and GC) and lipid-soluble choline (PtdCho and SM) in a healthy Norwegian population and to investigate the contribution from food groups and food items to this intake. Additionally, the associations between total choline intake and metabolites in one-carbon metabolism and lipid metabolism will be assessed.

Based on previously conducted dietary surveys where the average choline intake has been below the AIs set by both EFSA and NAM (6, 52), we hypothesize that the estimated mean choline intake in a healthy Norwegian population is lower than the established recommended AIs for American and European populations.

3. Materials and methods

3.1. Study population

To assess the intake of choline and all its forms in a Norwegian population, data from The Hordaland Health Study '97-'99 (HUSK2, https://husk.w.uib.no) in Western Norway was used. HUSK is a cooperative project between the University of Bergen, National Health Screening Service (now The Norwegian Institute of Public Health), and local health services. HUSK2 was the second study conducted in this project and included participants living in Hordaland (currently part of Vestland) born between 1925-27 or 1950-51. All subjects gave their written informed consent before participation. The study protocol was approved by the Regional Ethics Committee for Medical Research Ethics and by the Norwegian Data Inspectorate (57).

Data from 7016 HUSK2 participants was available for this thesis, whereas 6094 participants fully completed the food frequency questionnaire (FFQ) used for dietary analysis. Individuals missing data on choline intake were excluded (n = 922), and so were subjects missing measurements of plasma choline (n = 30). Furthermore, individuals with extreme energy intakes (<3.300 kJ or >17.500 kJ for males, and <3.000 kJ or >15.000 kJ for females) were excluded (n = 198). In addition, we excluded those with a self-reported daily alcohol intake >10 E% (n = 120). After exclusion, 5746 subjects remained and were considered eligible for analysis.

3.2. Baseline characteristics

Information on lifestyle, health behavior and medical history was obtained through self-administered questionnaires. Participants were defined as smokers based on self-reported smoking habits and if they had a serum cotinine level >85 nmol/L. Diabetes mellitus was defined according to preexisting self-reported diagnosis according to diagnostic criteria at the time of screening. Subjects were considered hypertensive based on self-reported use of antihypertensive medication.

The participants also attended a brief health examination and delivered a venous blood sample. The blood sample was taken non-fasting. Biochemical analyses of plasma levels of one-carbon metabolites and cotinine were performed by Bevital A/S, Bergen, Norway (http://www.bevital.no). Serum concentrations of total cholesterol (TC), high-density-lipoproteins (HDL) and TG were analyzed within 7 days at the department of Clinical

Chemistry, Ullevål University Hospital, Oslo using enzymatic methods. Low-density-lipoprotein (LDL) concentration was calculated from levels of TC, HDL-cholesterol and TG using the Friedewald equation. Weight and height were measured with light clothing, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

3.3. Dietary assessment

Data on dietary intake was obtained at baseline using a FFQ that was handed out as the participants attended their health examination (58). It was filled out at home and returned by mail to the HUSK project center. The semi-quantitative FFQ aimed to capture the participant´s habitual diet during the past year and is a slightly modified version of a previously described and validated FFQ (59, 60). It included 169 food items grouped according to a Norwegian meal pattern. The frequency alternatives ranged from once a month to several times per day, depending on food item. It included portion size alternatives in units eaten (e.g. slices, pieces) or household measures (e.g. spoons, cups, glasses, deciliter). In addition to food items, the FFQ included the nine most common single- and multivitamin supplements at the time of the study, but there were no specific questions on choline supplementation. Daily nutrient intakes were computed using the software system "Kostberegningsystemet" (KBS software, version 3.2.), developed at the Department of Nutrition, University of Oslo, Norway, which is primarily based on the official Norwegian food composition table (56).

3.4. Choline composition data

Calculations on choline intake includes the five individual choline forms, FC, GC, PC, PtdCho, and SM, and the sum of these given as total choline. Choline intake was quantified according to the values of choline and choline compounds established in the only available database, the USDA Database (54). From the 169-item FFQ, the content of choline and choline compounds was calculated from a total of 134 food items. Choline content in foods missing in the USDA Database were estimated using nutritionally equivalent foods. The 134 food items were categorized into 10 main food groups (dairy, drinks, eggs, fats, fish, fruit, grain products, meat, other, and vegetables), and subsequently into categories and subcategories based on nutrition similarities. A full overview of food items and categorization into subcategories, categories and food groups is provided in **Supplementary Table 1.**

3.5. Statistical analyses and Software

For baseline variables and dietary intakes, continuous variables are reported as means with standard deviations and categorical variables as counts with percent. The density method was used for adjusting dietary variables for self-reported energy intake, and values are reported as nutrient density (g/1000 kcal) or in energy percentage (E%) (61). This gives an overview of the dietary composition rather than the absolute intakes. The nutrient density method allows us to calculate the nutrient intake for an individual directly, and it is a familiar method that has been used in national dietary guidelines (61).

Choline intakes were adjusted for energy intake using the residual method. The residual method uses linear regression analyses with total energy intake as the independent variable and nutrient intake as the dependent variable (61). By computing residuals equal to the difference between expected intake predicted by total energy intake and the actual intake, we remove the variation caused by the energy intake, and the reported choline intake is thus relative to the individual energy intake. To calculate the percentage of contribution from food items and food groups, the intake of total choline or choline form from each food item or group was divided by the total intake of total choline or choline form and multiplied by 100.

To explore the relationship between one-carbon metabolites and choline intake, choline intake was modeled as a polynomial spline in a model with sex as an interaction term and adjusted for age, BMI and smoking. The same model was used to investigate the associations between lipid metabolites and choline intake.

Only TC and HDL-cholesterol were measured from serum samples. To calculate LDL-cholesterol, the Friedewald equation was used (*LDL-cholesterol = TC – HDL-cholesterol – (TG / 5*)). With this method, the concentration of VLDL-cholesterol is taken in consideration when estimating the concentration of LDL-cholesterol.

Statistical analyses were performed using R version 1.3.959 (The R Foundation for Statistical Computing, Vienna, Austria), and the packages within the *"tidyverse" ("dplyr", "ggplot2", "broom", "plyr", "ggthemes")* and the *"survival", "splines", and "janitor"* packages.

4. Results

4.1. Baseline characteristics

Baseline characteristics of the participants included for analysis are provided in **Table 2**. The participants are divided in groups across sex and birthyear. Of the total population, 2507 (43.6%) were men. There were 2786 (48.5%) men and women born in 1925-27, while the rest were born 1950-51. From here on, those born in 1925-27 will be referred to as "elderly", while those born in 1950-51 will be referred to as "middle-aged". The age ranged from 47 to 74 years at the time of data collection, with a mean of 59.8 years. The mean BMI was 25.7 (±3.8) kg/m^2 and was similar across sex and age groups. In the total population, 1566 subjects were defined as current smokers based on cotinine levels >85 nmol/L and self-reported smoking habits of cigarettes, cigars or pipe. Of the participants, 201 reported on having diabetes mellitus type 1 or 2, and 909 classified as hypertensive based on self-reported use of antihypertensive medication. Among the self-reported diabetic and hypertensive participants, respectively 87.6% and 85.7% were elderly. The mean serum concentration of TC in the study population was 6 (±1.1) mmol/L. The total population had a mean LDL-cholesterol of 4.3 (±1) mmol/L and a mean HDL-cholesterol of 1.3 (±0.4) mmol/L. The highest average concentrations of both TC, LDL and HDL were observed in elderly women. Mean concentration of TG across the study population was 1.8 (±1) mmol/L, with the lowest mean concentration measured in middle-aged women, and the highest in middle-aged men.

Table 2: Baseline characteristics of the study population across sex and birthyear.

	Total population	Female		Male	
Birthyear		**25-27**	**50-51**	**25-27**	**50-51**
n (%)	5746	1539 (26.8)	1700 (29.6)	1247 (21.7)	1260 (21.9)
BMI, kg/m^2	25.7 (±3.8)	26.2 (±4.4)	24.9 (±4)	25.9 (±3.1)	26.1 (±3.3)
Smokers[a]	1566 (27.3)	243 (15.8)	616 (36.2)	247 (19.8)	460 (36.5)
Diabetes[b]	201 (3.5)	96 (6.2)	7 (0.4)	80 (6.4)	18 (1.4)
Hypertension[c]	909 (15.8)	437 (28.4)	77 (4.5)	342 (27.4)	53 (4.2)
Serum lipids, mmol/L					
Total cholesterol	6 (±1.1)	6.6 (±1.1)	5.6 (±0.9)	5.9 (±1)	5.8 (±1)
LDL-cholesterol	4.3 (±1)	4.8 (±1.1)	3.9 (±0.9)	4.3 (±1)	4.3 (±0.9)
HDL-cholesterol	1.3 (±0.4)	1.5 (±0.4)	1.4 (±0.4)	1.2 (±0.3)	1.2 (±0.3)
Triglycerides	1.8 (±1)	1.8 (±0.9)	1.4 (±0.9)	1.8 (±1)	2.1 (±1.2)

Continuous variables are reported as means (±SD), and categorical variables are reported as counts (%).

[a] Defined according to self-reported smoking habits and serum cotinine levels >85 nmol/L.

[b] Defined according to pre-existing self-reported diagnosis.

[c] Defined according to self-reported use of antihypertensive medication.

4.2. Dietary intake

An overview of the dietary intake in the study population is provided in **Table 3.** The mean (±SD) reported daily energy intake was 1969 (±617.5) kcal. On average, males had a higher total energy intake than females, and middle-aged men consumed more energy than both elderly men and all women. Both unadjusted and energy-adjusted values of total choline intake are reported, and in the total population both means were calculated to 265.2 mg/d. However, a larger variation was observed for the unadjusted choline intake, as the choline intakes were positively correlated with energy intake. Middle-aged women had an unadjusted choline intake of 252.6 (±80.3) mg/d and a total energy intake of 1877 (±493) kcal/d, while elderly women had a choline intake of 227.2 (±77.1) mg/d and an energy intake of 1595 (±472) kcal/d. Likewise, both choline intake and energy intake were lower in elderly men than middle-aged men, who had the highest total choline and energy intake. Choline intake in middle-aged men was 321.9 (±93.4) mg/d and the energy intake was 2483 (±615) kcal/d, while elderly men had a choline intake of 271.6 (±85.2) mg/d and a total energy intake of 2037 (±559) kcal/d. However, the adjusted choline intake was similar across sex and age groups, and ranged from 263-270 mg/d.

Regarding macronutrient intakes, a mean of 50.3 E% was obtained from carbohydrates, 15.9 E% from protein, 31.8 E% from fats and 1.4 E% from alcohol. On average, the participants consumed 178 g/1000 kcal dairy per day, with the highest intake being observed in elderly women. Mean intake of eggs was 8.7 g/1000 kcal, and the mean fat intake was 15.1 g/1000 kcal. In general, women consumed more fruit than men, while the intake of grain products was similar across sex and age groups with a mean of 122.5 g/1000 kcal. Middle-aged participants consumed less fish, but more meat than elderly participants. The lowest mean vegetable intake was measures in middle-aged men, while elderly women consumed the most vegetables.

Table 3: Dietary intake in the total study population across sex and birthyear.

	Total population	Female		Male	
Birthyear		**25-27**	**50-51**	**25-27**	**50-51**
n (%)	5746	1539 (26.8)	1700 (29.6)	1247 (21.7)	1260 (21.9)
Choline[1], mg/d	265.2 (±90.1)	227.7 (±77.1)	252.6 (±80.3)	271.6 (±85.2)	321.9 (±93.4)
Choline[2], mg/d	265.2 (±55.9)	270.5 (±50.1)	263.2 (±55.3)	263.9 (±57.5)	263 (±61.3)
Energy intake, kcal	1969 (±617.5)	1595 (±472)	1877 (±493)	2037 (±559)	2483 (±615)
Macronutrient intake, E%					
Carbohydrates	50.3 (±6)	52.1 (±6.3)	49.5 (±5.9)	50.5 (±5.8)	48.9 (±5.6)
Protein	15.9 (±2.4)	16.1 (±2.5)	16.1 (±2.4)	15.9 (±2.3)	15.6 (±2.2)
Fat	31.8 (±5.5)	30.5 (±5.9)	32.4 (±5.3)	31.7 (±5.3)	32.8 (±5.1)
MUFA	10 (±1.9)	9.5 (±2)	10.3 (±1.8)	9.9 (±1.8)	10.5 (±1.8)
PUFA	6.8 (±2)	6.2 (±1.8)	6.9 (±2)	6.8 (±1.9)	7.3 (±2.1)
SFA	12.5 (±2.7)	12.3 (±3)	12.7 (±2.4)	12.4 (±2.8)	12.5 (±2.3)
Alcohol	1.4 (±1.9)	0.7 (±1.4)	1.5 (±1.7)	1.5 (±2.1)	2.3 (±2.2)

Food group intake, g/1000 kcal					
Dairy	178 (±109.4)	211 (±118.3)	153.8 (±99.3)	178 (±107.1)	169.6 (±103.2)
Drinks	464.2 (±246.4)	464.6 (±257.1)	526.7 (±267.9)	391 (±212.7)	452 (±209.6)
Eggs	8.7 (±6.5)	9.6 (±7.6)	8.6 (±5.8)	9 (±6.9)	7.4 (±5.1)
Fats	15.1 (±8.3)	13.3 (±7.9)	15.2 (±8.6)	15.5 (±7.6)	16.7 (±8.5)
Fish	43.2 (±24.4)	45.8 (±26.3)	38.7 (±21.3)	52.7 (±25.9)	36.8 (±20.8)
Fruit	127 (±85.3)	150.6 (±100.3)	136.2 (±84.1)	115.4 (±74)	97.1 (±64.2)
Grain products	122.5 (±31.4)	123.6 (±34.8)	119.8 (±29.1)	123.3 (±32.5)	123.8 (±28.9)
Meat	49.4 (±23)	39.4 (±19.6)	56 (±23.4)	45 (±20.8)	57.3 (±22.8)
Other	6.3 (±7.8)	5.5 (±7.4)	7.4 (±8.8)	5.7 (±7.1)	6.4 (±7.1)
Vegetables	166.7 (±82.4)	184.1 (±85.8)	171.2 (±85.5)	170.4 (±75.9)	135.7 (±71.3)

Continuous variables are reported as means (±SD) and categorical variables are reported as counts (%).

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

[1]Unadjusted values of total choline intake.

[2]Energy-adjusted values of total choline intake.

4.3. Intake of choline

Energy-adjusted intakes of total choline and individual choline forms are presented in **Table 4.** As already mentioned, the mean energy-adjusted choline intake in our study population was 265.2 (±55.9) mg/d. Elderly women had a slightly higher intake of total choline compared to the other groups, with an intake of 270.5 (±50.1) mg/d, however, adjusted intakes were similar between groups. PtdCho was the major contributor to the total intake of choline and constituted 43.5% of the total intake. Thereafter, 26% of the total intake was in the form of FC, and 22% came from GC. PC and SM only contributed to a small fraction of the total intake, more specifically with 4.7% and 4.4% each respectively.

Table 4: Mean energy-adjusted intakes of total choline and choline forms (mg/d) in the study population, across sex and birthyear.

	Total population	**% of total choline**	**Female**		**Male**	
Birthyear			**25-27**	**50-51**	**25-27**	**50-51**
n (%)	5746		1539 (26.8)	1700 (29.6)	1247 (21.7)	1260 (21.9)
Total choline	265.2 (±55.9)	–	270.5 (±50.1)	263.2 (±55.3)	263.9 (±57.5)	263 (±61.3)
Free choline	69.1 (±14.4)	26.0	69.9 (±12.4)	68.3 (±14.3)	68.6 (±14.6)	69.5 (±16.5)
Glycerophosphocholine	58.4 (±22.3)	22.0	62.8 (±19.6)	52.8 (±20.5)	59.9 (±22.2)	59.3 (±25.9)
Phosphocholine	12.5 (±4.7)	4.7	13.4 (±4.2)	12.5 (±4.9)	12.1 (±4.4)	11.7 (±5.1)
Phosphatidylcholine	115.5 (±33.7)	43.5	114.6 (±30.7)	119.6 (±32.2)	114 (±35.5)	112.7 (±36.9)
Sphingomyelin	11.8 (±3.3)	4.4	12 (±3.1)	11.8 (±3.1)	11.7 (±3.4)	11.7 (±3.6)

Continuous variables are reported as energy-adjusted means (±SD), and categorical variables are reported as counts (%).

The density plot in **Figure 5** shows the mean choline intake for men (263.4 mg/d) and women (266.6 mg/d) and the distribution around the means. The majority of the population group studied had a total choline intake between 150-400 mg/d. The lines indicating the AIs estimated by EFSA and NAM show that most subjects in our study population had an average intake below the recommendations. Among women, 55 (1.7% of all women) had an intake above EFSA´s recommendation of 400 mg/d, while 58 men (2.3% of all men) achieved this AI. Only one man had an intake above 550 mg/d, which is the recommended AI for men in the US. Regarding the NAMs AI for women, 39 (1.2% of all women) had an intake above the requirement of 425 mg/d.

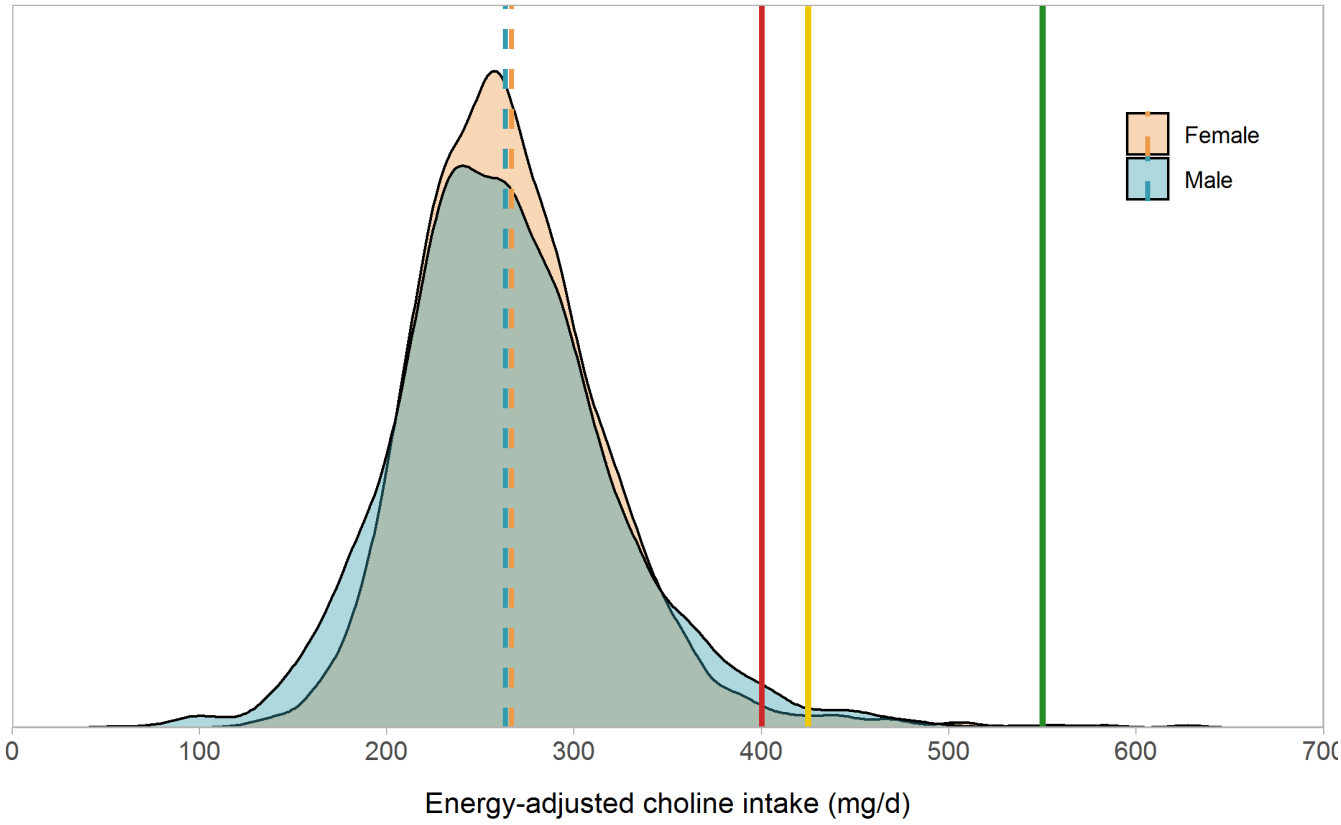

Figure 5. The distribution of mean choline intake in the study population compared to recommended AIs by NAM and EFSA. The dotted blue line indicates the mean energy-adjusted total choline intake for men, while the orange dotted line indicates the mean energy-adjusted total choline intake for women. The solid red line indicates the AI for men and women set by EFSA. The solid yellow line indicates the AI set by NAM for females while the solid green line indicates the AI set by NAM for males.

4.4. Food groups contributing to choline intake

The contribution to choline intake from different food groups is depicted in **Figure 6.** Out of the ten main food groups, dairy, including milk, yoghurt and cream products, was the major source of total choline in the study population and constituted of 19.2% of the total intake. The second major food group contributing to total choline was vegetables (17.2%), and thereafter eggs (15.3%). Most of the water-soluble choline forms FC, GC and PC came from vegetables, fruit, dairy, grain products, drinks and fish. Lipid-soluble PtdCho was mostly obtained through eggs (33.8%), while the intake of SM primarily came from dairy, eggs, fish, grain products, and meat. The major sources of water-soluble and lipid-soluble choline differed. More water-soluble choline was obtained from drinks and plant-based sources, such as vegetables and fruits, whereas fats and animal sources, including eggs and meat did not contribute much to the intake of FC, GC and PC, but were good sources to lipid-soluble choline. Dairy, grain products and fish were sources to both water-soluble and lipid-soluble choline in this population. Food groups with little contribution to the total choline intake were fats and other foods, including sugar, sweets, and snacks.

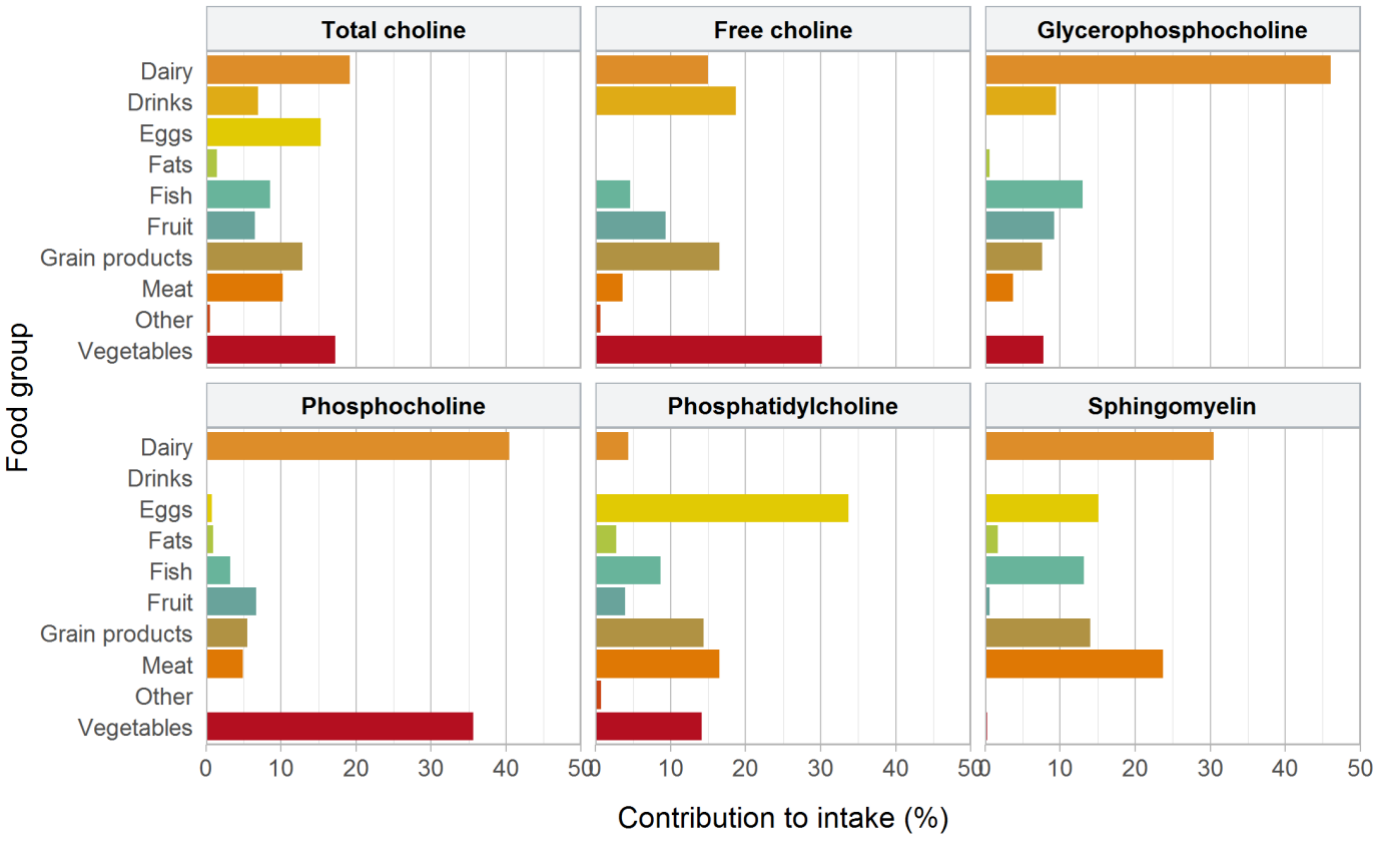

Figure 6. Food groups contributing to the intake of total choline and all choline forms in percentage of total intake of total choline or individual form.

The contribution from food subcategories is presented in **Table 5** and **Supplementary Table 2a-e**, where the ten major contributing food subcategories are ranked for total choline (**Table 5**) and choline forms (**Supplementary Table 2a-e**). Eggs was the major contributor to the total choline intake, whereas low-fat milk was the second largest source to total choline in our population. Potatoes, leafy vegetables, and wholegrain bread were significant sources to choline as well. GC and PC were mainly acquired from low fat milk (31.7% and 26.7% respectively). Leafy vegetables, potatoes, and other vegetables were also large contributors to the PC intake, while the second and third contributors to the intake of GC were fish products and fatty fish. FC was primarily obtained from coffee, potatoes, low-fat milk, wholegrain bread, and leafy vegetables. Besides being mainly obtained from eggs, some of the intake of PtdCho also came from meat spread, leafy vegetables, pastries, fish products, and wholegrain bread. The food subcategories contributing to the SM intake were mainly eggs, low-fat milk, poultry, fish products and fish spread, white cheese and wholegrain bread.

Table 5: Primary food subcategories contributing to the intake of total choline.

Rank	Food item	Contribution, %	Cumulative contribution, %
1	Eggs	15.3	15.3
2	Low fat milk	11.8	27.1
3	Potatoes	6.3	33.4
4	Leafy vegetables	5.7	39.1
5	Wholegrain bread	5.2	44.3
6	Fish products	4.8	49.1
7	Coffee	4.3	53.4
8	Fresh fruit	4.2	57.6
9	Meat spread	3.7	61.3
10	Fish spread	3.5	64.8

4.5. Associations between choline intake and circulating biomarkers of metabolism

4.5.1. Associations between choline intake and one-carbon metabolites

Plasma concentrations of metabolites involved in one-carbon metabolism across sex and birthyear are presented in **Table 6**. Betaine, choline, DMG, methionine, and TML concentrations were higher in men than women, while concentrations of DMG and serine were higher in women. Mean concentrations of Hcy, cystathionine, cysteine, and TMAO were higher in elderly men and women than in middle-aged participants. In the total population, mean (±SD) concentration of plasma choline was 9.9 (±2.3) µmol/L, and the highest average mean was measured in elderly men while middle-aged women had the lowest mean plasma choline.

Table 6: Plasma concentrations of one-carbon metabolites in µmol/L across sex and birthyear.

	Total population	**Female**		**Male**	
Birthyear		**25-27**	**50-51**	**25-27**	**50-51**
n (%)	5746	1539 (26.8)	1700 (29.6)	1247 (21.7)	1260 (21.9)
Betaine	37.5 (±10.5)	35.2 (±8.9)	32.5 (±9.1)	42.5 (±9.9)	42.4 (±10.6)
Choline	9.9 (±2.3)	9.8 (±2.3)	9.1 (±1.9)	11 (±2.5)	9.9 (±2.1)
Cystathionine	0.3 (±0.8)	0.4 (±0.3)	0.2 (±0.2)	0.4 (±0.5)	0.3 (±1.6)
Cysteine	288.2 (±31.6)	307.4 (±27.4)	265.4 (±24.3)	305.4 (±27.6)	278.3 (±23.3)
DMG	4.6 (±2.5)	4.5 (±4)	4.4 (±1.2)	4.9 (±2.3)	4.9 (±1.3)
Glycine	261 (±76.7)	279.9 (±87)	282.3 (±86.8)	234.6 (±51.5)	234.5 (±47.8)
Homocysteine	11.6 (±4.3)	12.2 (±3.9)	9.6 (±3.3)	13.7 (±5.3)	11.4 (±3.6)
Methionine	28.5 (±8.4)	27.2 (±8.5)	27.5 (±7.4)	29.9 (±8.8)	30.2 (±8.4)
Serine	116.6 (±24.9)	116.3 (±25.5)	123.4 (±26.9)	111 (±22)	113.4 (±21.8)
TMAO	9.3 (±16.9)	10.8 (±20)	6.1 (±9.8)	13.3 (±21.3)	8 (±14.1)
TML	0.6 (±0.3)	0.6 (±0.3)	0.5 (±0.3)	0.7 (±0.3)	0.7 (±0.3)

Continuous variables are reported as means (SD), and categorical variables are reported as counts (%).

The associations between dietary choline intake and concentrations of one-carbon metabolites are investigated and presented in **Figure 7.** Betaine levels in response to choline intake varied between men and women. Little change was observed in betaine concentrations across choline intake in women, but betaine was increased in men with a low or high choline intake. Plasma choline in men increased with increasing intakes of choline, while for women, plasma choline decreased up to an intake of about 200 mg/d, before increasing with higher choline intakes. Levels of cystathionine and cysteine were positively associated with choline intake, but the curves flatten out at the highest intakes. DMG level in women increased with higher choline intakes before slightly decreasing at an intake >400 mg/d. In men, DMG concentration in relation to choline intake was similar to the associations observed for betaine and was slightly increased in men with the lowest choline intake and increased with higher intakes. Plasma concentrations of Hcy, glycine, and serine were negatively associated with choline intake in both men and women. On the other hand, methionine and TML concentrations were positively associated with choline intake. TMAO increased with a choline intake up to about 300 mg/d but was decreased in participants who exceeded this intake.

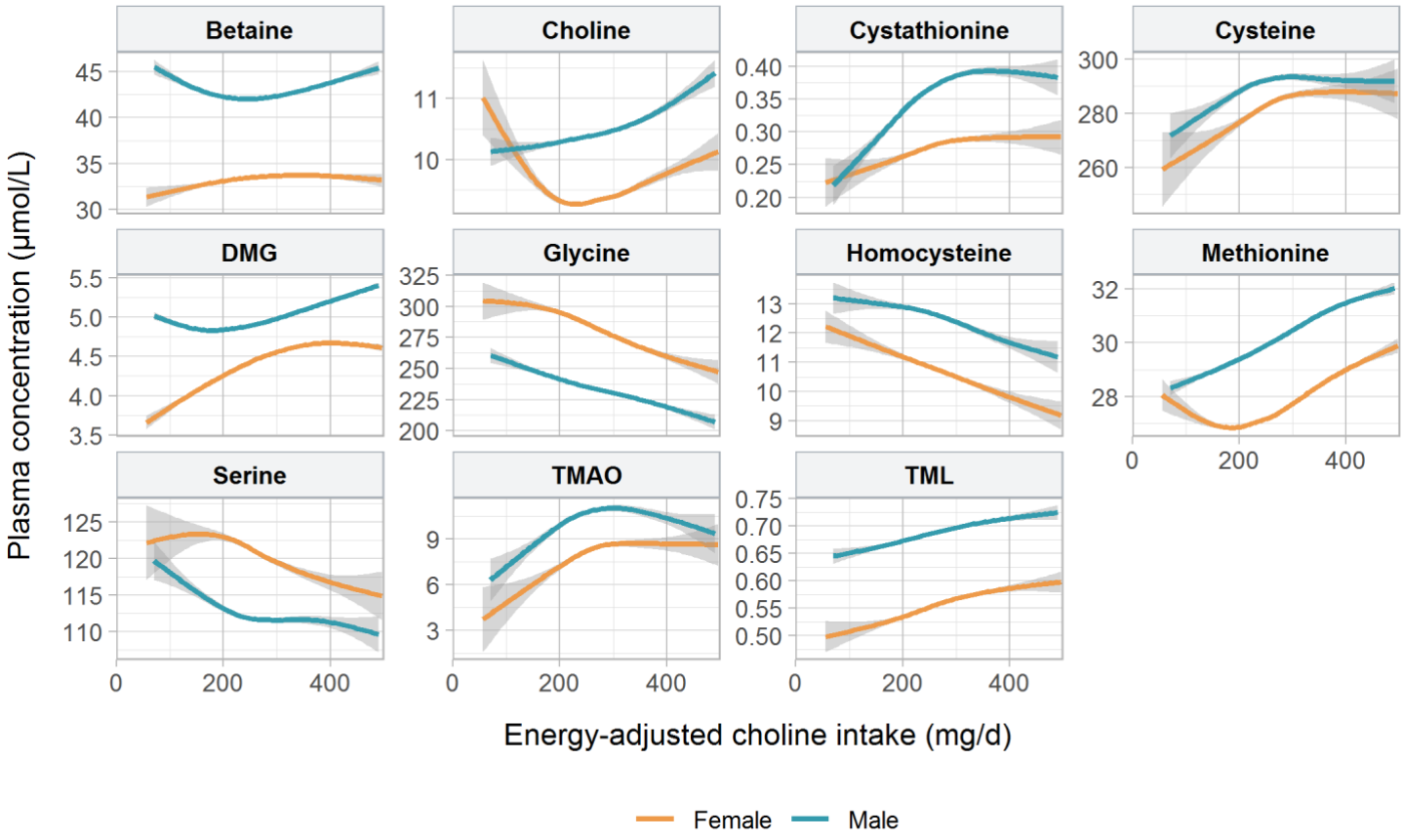

Figure 7. Associations between dietary choline intake and concentrations of one-carbon metabolites. The gray area indicates the standard deviation.

4.5.2. Associations between choline intake and lipid metabolites

A positive association was observed between choline intake and TC concentrations in both men and women (**Figure 8**). The variation was larger in women and the concentration increased in women with a choline intake up to about 300 mg/d. In men, only a small increase in TC was observed in those with a higher choline intake. The associations between choline intake and concentrations of both TC and LDL-cholesterol were similar in men and women, and LDL-cholesterol was positively associated with choline intake in both sexes. Like the TC in women, LDL-cholesterol increased with an intake up to about 300 mg/d, before decreasing at higher intakes, while a smaller increase was observed in men. In general, HDL-cholesterol was higher in women than men. In women, choline intake up to about 250 mg/d was associated with higher HDL-cholesterol, while in men HDL-cholesterol decreased with higher choline intakes. TG concentration was higher in men than women, and a positive association with choline intake was detected in both sexes.

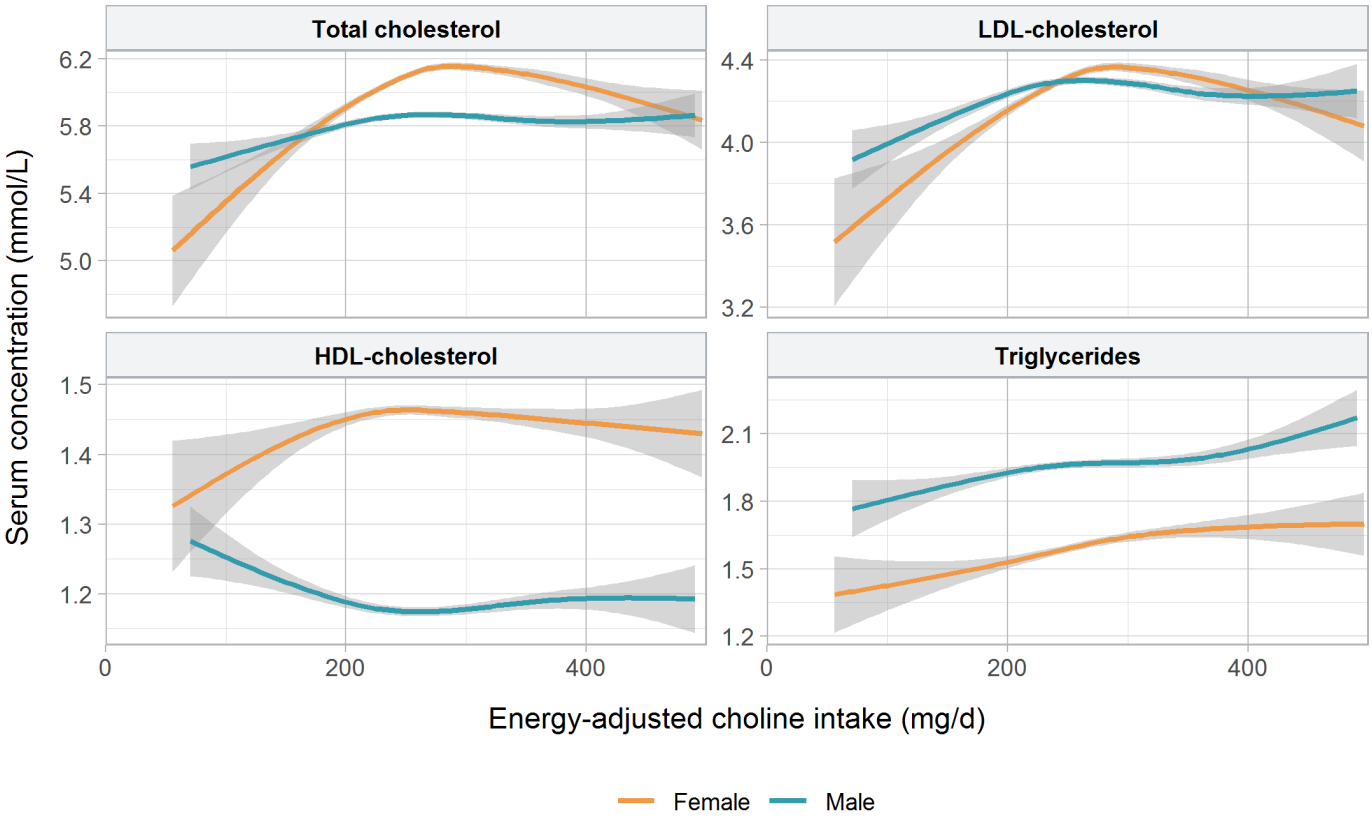

Figure 8. Associations between dietary choline intake and serum levels of lipid metabolites. The gray area indicates the standard deviation.

5. Discussion

5.1. Summary of main findings

This study aimed to investigate the dietary intake of total choline and individual choline forms in a healthy Norwegian population. The mean energy-adjusted total choline intake was 265.2 mg/d for the whole study group, 263.4 mg/d for men and 266.6 mg/d for women. The observed means are below the recommended AIs set by both EFSA and NAM. Choline was mainly obtained from the lipid-soluble form PtdCho. The main foods contributing to the intake of total choline were eggs, low-fat milk, potatoes and leafy vegetables, wholegrain bread and fish products. Associations between one-carbon metabolites and choline intake were also investigated. In this study population, choline intake was positively associated with methionine, cystathionine, cysteine, TML, TMAO, and DMG, as well as plasma choline in men, and inversely associated with plasma concentrations of Hcy, glycine, and serine. Concentrations of TC, LDL-cholesterol and TG were positively associated with a higher choline intake in both men and women. HDL-cholesterol increased in women with higher choline intakes, but a negative association was observed in men.

5.2. Discussion of findings

5.2.1. Dietary intake and sources of total choline

Choline intake has been estimated in men and/or non-pregnant, non-lactating women in China (48), Mexico (49), Taiwan (50), New Zealand (51), USA (35, 46, 62-65), and in eight European countries (52). The estimated choline intakes from these studies are similar to the mean choline intake estimated in our Norwegian study population. In China, Mexico, Taiwan, and New Zealand, the choline intake ranged from 178-289 mg/d in women and 318-372 mg/d in men, while in the US the observed estimated intakes ranged from 258-323 mg/d in women and 302-405 mg/d in men. In European countries, the estimated intakes ranged from 291-374 mg/d in men and women. Based on their findings, all studies have drawn the conclusion that most individuals probably do not achieve the AIs of choline set by NAM and EFSA.

However, the resulting choline intake estimates from dietary surveys conducted around the world cannot be directly compared due to different methodologies. First of all, the studies have used different dietary assessment methods, including 24 hours recall, FFQ and several days of food records. There are also differences in age classes, number of participants, number of reporting days, method for data collection, and statistical methods. Moreover,

some studies included energy-adjusted the total choline intake, while others have reported the unadjusted intake. This could explain some of the observed difference in the average intakes between men and women, since men in general have a higher total energy intake and therefore also a higher total choline intake. Choline intake could also be affected by ethnicity and/or race, but it has been observed that variations in choline intake largely rely on energy intake (63). Prior to energy-adjustment, we observed that choline intake correlated with total energy intake in our study population, however, it was similar across age and sex groups after energy-adjusting. Moreover, even though all studies have used values of choline in foods from the USDA Database, there are today two published releases of the database (44, 66), and new calculations of choline content in foods have replaced some of the old values in the updated release.

Interestingly, the highest mean choline intakes in both men and women have been measured in Sweden, the neighbor country of Norway. Choline intake was estimated from a Swedish national dietary survey and calculated to 468 mg/d in men and 374 mg/d in women (52, 53). The survey included 1420 healthy adults who completed a 4-d self-administered food record, a method that differs remarkably from the dietary assessment method used in this study. Additionally, the choline intake in Sweden was not energy-adjusted, and these two factors could explain some of the observed difference between choline intake in Sweden and Norway. Nevertheless, it could be an actual difference in the dietary intake that affects the choline intake. However, the only observed differences in macronutrient distribution was that the Norwegian population had a higher intake of carbohydrates (6.7 E% more) than the Swedish population, while in Sweden, more energy was obtained from fats (2.4 E% more). Total energy intake and E% from protein, which is a significant source of choline, were similar between the two countries. The difference in dietary intake cannot in itself explain why the measured intake was higher in Sweden than in Norway, and possible reasons would be interesting to investigate further.

Data suggests that most people do not achieve the recommended AIs, but in a study that measured the exact amount of food consumed, the measured choline intake met or slightly exceeded the current AIs in all participants (43). The study was conducted in 16 men and 16 women, who consumed a freely chosen diet were all meals were weighed before and after serving. The mean measured choline in their diets was 631 mg/d in men and 443 mg/d in women, means that exceed the AIs set by both NAM and EFSA. What was observed in this

study suggests that the recommended AIs are close to the actual dietary intake, which contradicts findings from most dietary surveys. Considered the ubiquitous distribution of choline in foods, most healthy individuals with a varied diet should theoretically be able to consume adequate amounts to prevent choline deficiency. However, this theory is contradicted by the estimated means in dietary surveys worldwide. The adequacy of the current AIs and the validity of studies in choline intake and the dietary assessment methods used should therefore be considered.

Food sources of total choline

Eggs were the main contributor to the total choline intake in this study population. Eggs are also one of the foods item that contains most choline per weight of unit, after beef liver and chicken liver (55). However, compared to beef and chicken liver, eggs are more usually eaten on a daily basis and in larger quantities. In a cohort study from the US, egg consumption was significantly associated with higher usual intakes of choline in adults, and consuming eggs on a daily basis almost doubled the intake (46). Other protein-rich foods like meat, poultry and seafood also increased the choline intake, but not as much as the intake of eggs did. After eggs, the second largest source of choline in our population was low-fat milk, which was also the primary contributor to GC and PC, and the second and third largest source of SM and FC, respectively. Milk is not considered a particularly rich source of choline, containing under 20 mg of choline per 100 g (54). However, it has several times been reported to be a major contributor since it is usually consumed on a daily basis and it is a good source of both water-soluble and lipid-soluble choline (6, 7, 51, 65).

In addition to eggs and low-fat milk, potatoes, leafy vegetables, and wholegrain bread were also important contributors to the total intake of choline in this study. The major food contributors vary between countries, and some of the variation could be explained by different dietary patterns, cultures and ethnicity, in addition to possibly being affected by how the major food groups are defined. In studies from China and Taiwan, choline was primarily obtained from eggs, soy products, fish, and meat (48, 50). In New Zealand, Europe and the US, more choline is obtained from grain products, dairy, and coffee, in addition to meat, poultry, fish, eggs, and composite dishes (51, 52, 62). Despite some differences, it seems to be some agreement between countries regarding the major food groups contributing to choline, which is supported by our results. However, animal food sources such as meat, poultry and fish, were not among the five largest contributing food subcategories in this study population.

The Norwegian yearly consumption of meat per person is lower than in many European countries, and especially lower than in the US, and this could explain why animal products did not contribute as much as would be expected (67). On the other side, wholegrain bread, potatoes and milk were among the most important contributors, which could be explained by a traditionally high consumption of potatoes and bread in Norway.

5.2.2. Dietary intake and sources of choline forms

The main choline form consumed in this study population was PtdCho, which accounted for nearly half of the total intake. The intake of individual choline forms have been investigated in New Zealand (51), Canada (68), and the US (65, 69). All studies agree that the main choline form consumed in a general adult diet is PtdCho, and lipid-soluble choline forms usually contribute to about 45-60% of the total choline intake. After PtdCho, the water-soluble choline forms FC and GC contributed with 26% and 22% each respectively, and these water-soluble forms are the second and third most consumed forms in other investigated populations as well (51, 65, 68, 69).

Food sources of individual choline forms

We only identified studies from the US that described the contribution of food items to all individual choline forms (35, 69, 70). Our results are similar to their findings, however, there are some differences worth noticing. Both in the US and in Norway, FC was mainly obtained from coffee, potatoes, and milk, while the intake of GC was mainly acquired from milk, fish, coffee, and potatoes. However, the main sources of PtdCho in the US were eggs, beef, chicken, pork, and liver, while in the Norwegian population, much of the PtdCho intake was obtained from consumption of leafy vegetables, pastries, wholegrain bread, and potatoes, in addition to eggs and meat spread. It seems that the intake of PtdCho from animal food sources is larger in the US than in Norway, which could be explained by a generally lower meat consumption in Norway (67).

5.2.3. Associations with plasma one-carbon metabolites and serum lipids

Associations between choline intake and plasma concentrations of one-carbon metabolites and serum lipids were investigated. Our findings show that dietary choline intake affects the concentration of several metabolites involved in one-carbon metabolism. The concentration of metabolites was dependent of sex, and some of the observed changes in plasma concentration of metabolites across choline intake varied between men and women. Since the blood samples were taken non-fasting, it must be considered that the concentration of metabolites could have been affected by prandial status. For instance, plasma choline level is known to increase 10-15% after a meal, and is particularly responsive to large intakes of dietary sources of choline (21). Nevertheless, we observed that plasma choline was positively associated with a long-term intake of choline in men, while the association was unclear in women. However, low plasma choline has not been correlated with susceptibility to develop organ dysfunction, and it might not be a sufficient predictor of choline deficiency-related organ dysfunction (10). Moreover, the only food item directly associated with plasma choline in this study population was eggs (data not shown), which indicate that plasma choline might be a good predictor of egg consumption, but not of total dietary choline intake (71).

The relationship between serum cholesterol and TG levels, and CVD is well established. But the role of phospholipids, including choline phospholipids, and their effect on CVD risk is less investigated (72). We observed that concentrations of TC, LDL-cholesterol, and TG were increased in participants with a high choline intake. A proposed mechanism behind is that choline intake increases the availability of PtdCho for VLDL-assembly and secretion, which subsequently increases the export of lipids from the liver (73). Concentration of TG has been observed to increase after only two weeks of PtdCho supplementation (74). However, several studies have found no associations between choline intake and serum cholesterol, and the relationship is unclear (10, 74, 75). Nevertheless, our model adjusted for sex, age, BMI, and smoking show that choline intake could possibly contribute to increased concentrations of TC, LDL-cholesterol and TG, which could be a potential CVD risk factor.

On the other hand, choline has been observed to have a Hcy-lowering effect. High plasma Hcy is associated with increased risk of CVD and cardiovascular mortality, and this could potentially benefit the CVD risk profile (14). We found an inverse association between choline intake and plasma levels of Hcy in both men and women, which has been previously observed in several studies (10, 35, 65, 75). As a source of betaine, choline intake increases

the potential for remethylation of Hcy to methionine and could thereby reduce Hcy levels. Supplementation of betaine has been used to lower Hcy levels in people with hyperhomocysteinemia (37), and can decrease Hcy levels by up to 20% in a general population (76). But betaine supplementation has been associated with a negative change on blood lipids by causing an increase in the LDL-cholesterol, an association that has not been observed in studies in choline supplementation (74, 76). This raises the question whether choline supplementation could be a more suitable supplement than betaine in the treatment of hyperhomocysteinemia. However, taking our observations of an increase in both LDL-cholesterol and TG in consideration, more studies are needed before drawing any conclusions.

The association between total choline intake and plasma Hcy concentration has in another study been observed to be stronger in men than women, possibly due to a higher *de novo* choline synthesis in women (65). Moreover, the concentration of Hcy can be affected by a range of other factors, including age, sex, smoking, alcohol and caffeine consumption, blood pressure and the use of antihypertensive medication (77, 78). It has also been observed that the Hcy-lowering effect of water-soluble and lipid-soluble choline forms differs, as the lipid-soluble forms seem to be positively associated with Hcy (35, 37). SM and PtdCho have also been associated with increased risk of cardiovascular events (79). These findings raise the question whether the observed association is linked to choline in itself, or other components of food items high in lipid-soluble choline. Dietary sources of lipid-soluble choline include eggs, beef, chicken, and milk. These food sources also tend to be high in cholesterol and the TMAO precursor carnitine, which have already been related to CVD risk via different pathways (80). Carnitine and choline are both dietary precursors of TMAO, which have been positively associated with cardiovascular events, and levels have been observed to increase after choline intake (81, 82). As would be expected, we found a positive association between TMAO and choline intake, but only up to about 300 mg/d. Due to the relationship between choline intake and TMAO and CVD risk, Meyer et.al. investigated the association between choline intake and incident CVD in a meta-analysis, but no associations were found (19). Further research is needed to investigate the relationship between choline intake, the intake of food items contributing to choline and CVD.

5.3. Methodological considerations

5.3.1. Study design

One of the biggest advantages of this study is the large sample-size of free-living healthy individuals. We have analyzed dietary consumption in two age groups, both middle-aged and elderly. Since dietary intake often depends on age, this is another advantage. However, the population is not representative for younger age groups, pregnant or lactating women. Another strength is that the intake of all individual choline forms has been analyzed. The metabolic fate of the individual choline forms differs, and could thus impact growth, development and health differently (6). Because the intake of choline forms depends on the dietary pattern of an individual, it is necessary to investigate the intake of the forms and their health effects individually.

5.3.2. Choline intake measurements

Unfortunately, the Norwegian food composition table does not include values for choline, and all calculations of choline intake are therefore based on values from the USDA Database (54, 56). This is a source of discrepancies, as the choline composition in common American food items may not adequately reflect the choline content in local Norwegian foods. Country-to-country variation in choline content in foods could be significant, since choline content can differ due to manufacturing and preparation methods, and due to natural variations caused by e.g. season and geographical location (54). Before the actual choline content in Nordic food items is measured, any interpretations of the estimated choline intake in Norway must be done with caution.

Additional choline intake from supplements has not been considered in this analysis. Most mainstream multivitamin supplements do not contain choline, and very few consume choline as a single-nutrient supplement. Moreover, analyses from the US suggest that multivitamin or mineral supplements do not significantly affect choline intake at a population level (83).

5.3.3. Dietary assessment method

Dietary data was obtained using a self-administered FFQ, a method that has both advantages and limitations. First of all, this is a method with low cost and low participant burden, and it is therefore frequently used for large cohort studies (84). Compared to short-term dietary instruments such as 24 hours recall and food records, an FFQ can capture the long-term food intake and dietary patterns over time and avoids some of the day-to-day variation in dietary

intake (84). This makes the FFQ less prone to random measurement errors that increase the variation in the data. However, since the FFQ was only filled out at baseline, it fails to capture possible dietary changes over time. Additionally, the pre-determined food list of 169 items may not have covered the actual dietary intake of all participants. This especially accounts for participants with dietary habits that differ from a typical Norwegian diet, e.g. immigrants, since the FFQ used was designed according to a Norwegian dietary pattern. This might have caused underestimation of the total energy intake, which could have led to exclusion from the analysis or have negatively affected the estimated choline intake. On the other hand, the specified food list allows us to capture the intake of foods that are not so frequently consumed, whereas some foods may contain significant amounts of choline, such as beef liver or chicken liver (54).

The main limitation of a FFQ is that it suffers from systematic measurement errors related to methodology, particularly due to the fact that the data is self-reported (85). Systematic errors, also called bias, are a type of measurement errors in which measurements consistently depart from the true value, often in the same direction. It is often observed as a systematic under- and over-reporting of food intakes, affected by social desirability and the desire to present oneself positively (86). “Less healthy” foods tend to be underreported compared to foods considered “healthy”. This could have caused significant errors in our analysis, as foods considered “less healthy” often include red meat, eggs, and fats, which are rich in choline. Additionally, since nearly all food items and drinks contain energy, every small or large error reported by the participants will add up and cause larger errors in the total energy intake estimation (86). Because of its related measurement errors, nutrient intakes estimated from a FFQ are recommended to be energy-adjusted. By energy-adjusting choline intake and reporting dietary intakes as g/1000 kcal or in E%, we might have been able to decrease the potential errors in our results (84).

The FFQ used in this study has not been validated for the intake of total choline or choline forms, and its ability to capture the actual choline intake cannot be evaluated. FFQs should be validated in the population and for the nutrient of interest, which makes this a limitation of our study (6). A tendency for higher choline intake estimates from FFQs compared to 24 hours recalls has been reported in validation studies from the US (87, 88). This could partly be explained by the systematic measurement errors that often occurs in dietary intake estimates from FFQs. Choline is abundant in many foods such as eggs, meat, leafy vegetables,

legumes, and dairy, and because of the set food list in FFQs, participants might overreport the variation in their diet, adding more food sources of choline to their reported habitual diet. Moreover, a study in 32 men and women found that the self-reported intake from a 3-d food record significantly underestimated the actual choline intake measured in their diet (43). When calculating the choline content in a diet they had freely chosen by weighing all meals before and after consumption, the choline content was measured to be 25% higher than what the participants had reported in their food records. However, the difference was non-existent after adjusting for energy intake, which suggests that an underestimation of the total energy intake also caused underestimation of choline and other nutrients. This proves that measurement errors can be significant, and it is one of the biggest disadvantages of using a self-reported dietary assessment method. Based on this, it must be considered that the estimated choline intake in our Norwegian study population might be underestimated compared to the actual intake.

5.3.4. AI as dietary recommendations

If current recommended AIs are valid, dietary assessments indicate that choline intake in Norwegians and other populations investigated is insufficient. This issue must then be considered as a possible significant health concern, since a suboptimal choline intake can lead to e.g. impaired liver function, fatty liver, or muscle dysfunction. However, the recommendations by EFSA and NAM are based on few studies and limited evidence, particularly the first AIs published by NAM, which are based on only one single study in men. NAM and EFSA agree that current data on choline intake is insufficient to establish an EAR, and thus have only established AIs. The AI is defined as a recommended average daily nutrient intake level, that is based on observed mean nutrient intakes or experimentally derived intake levels that are assumed to be adequate (89). Compared to an EAR, the AI is a guide about an appropriate nutrient intake level, rather than a requirement, and it should be higher than the unknown EAR. To establish an EAR, the intake that meets the nutrient requirement of half of the healthy individuals in a life stage and sex group must be established and it requires much more data than what is needed for establishing AIs. Given the definition of the AI, we cannot determine the prevalence of inadequate nutrient intakes in a population based on it (89). A group mean intake above the AI will assume low prevalence of inadequate intakes, while an intake below the AI not necessarily indicates inadequacy in the population. Therefore, we cannot draw any conclusions of the inadequacy of choline intake in the Norwegian population investigated before an EAR is established.

5.4. Implications and future perspectives

Based on our findings, dietary intake of choline in Norway does not meet the current dietary requirements set for the European nor the American population. In our study population, approximately 2% of the participants had an intake above the AI of 400 mg/d set by EFSA, while only 0.7% achieved the AIs set by NAM. If current AIs and the estimated choline intake are valid, strategies to increase choline intake on a population level should be assessed in Norway, as well as in other countries where the estimated choline intake has been below the AIs. One strategy is public education in foods that are rich in choline. Education in how to achieve the daily AIs should particularly be provided for pregnant and/or lactating women. Since choline is vital for a healthy development of the child, dietary requirements increase during these periods, and the majority of women today do not meet the AIs. Specific strategies to increase an individual´s choline intake could be to recommend a higher egg and milk consumption, and/or choline supplementation if recommended intake cannot be achieved through the diet. Fortification practices could be an additional strategy to increase the intake across a population. If applied, it should be considered to fortify staple products that are widely available and cost-efficient, e.g. grain products or dairy products.

Without an estimated EAR, it is not possible to interpret whether the low choline intake can contribute to a suboptimal health status. There are some concerns about the methods used to determine the current AIs, and they should therefore be reviewed. Additionally, a dietary assessment method validated for choline should be used in future studies to obtain more refined estimates on choline intake. Eventually, recommended daily intakes should be estimated for Norway and other Nordic countries, and more data on the choline intake in these populations must be obtained to achieve this. To improve the quality of the intake assessments, the choline content in Nordic food items should be calculated and added to the Norwegian food composition table.

6. Conclusion

To our knowledge, this is the first study to assess the intake of total choline and all choline forms in a healthy Norwegian population. To conclude, we found that the average intake of choline was below the recommended AIs for adults. Most choline was consumed in the form of PtdCho, whereas most of it was obtained from eggs, meat, grain products, and vegetables. Overall, the main contributors to total choline intake were eggs, low-fat milk, potatoes, leafy vegetables, and wholegrain bread. Our findings suggest that choline intake affects the concentrations of most metabolites involved in one-carbon metabolism, and supports that choline has a Hcy-lowering effect. However, choline intake was positively associated with concentrations of LDL-cholesterol, TC, and TG in both men and women, and could potentially have a negative impact on health outcomes being related to such a lipid profile in healthy adults.

Our findings are important considering the observed possible inadequate choline intake in Norway. To further investigate this issue, studies in choline intake in Norway and other Nordic countries are warranted, in addition to a database of choline content in Nordic foods. Eventually, recommended choline intake for Nordic countries should be estimated and the validity of the current AIs should be reviewed, so that the actual prevalence of choline deficiency can be assessed.

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Supplements

Supplementary Table 1: Grouping of food items into subcategories, categories, and food groups.

Food group	Category	Subcategory	Food items
Grain products	Bread	White bread	White bread
			Bread <50% whole wheat
		Wholegrain bread	Bread >50% whole wheat
		Other bread	"Lefse"
			Chapatti
			Taco
			Flatbread
			Crispbread
	Pastries	Buns	Waffles
		Biscuits	Biscuits
		Other pastries	Other pastries
	Other grain products	Pizza	Pizza
		Cereal	Cereal
			Oatmeal
Vegetables	Potatoes		French fries
			Fresh potatoes
	Fresh vegetables	Root vegetables	Carrot
			Turnip
		Leafy vegetables	Cabbage
			Cauliflower
			Broccoli
		Other vegetables	Leek
			Onion
			Tomato
			Bell pepper

			Spinach
			Parsley
			Vegetable mix
			Unspecified vegetables
		Canned vegetables	Pickled vegetables
			Other canned vegetables
Fruit	Fresh fruit		Citrus fruit
			Apple
			Pear
			Unspecified fruit
	Juice		Juice
	Other fruit	Conserved fruit/berries	Jam
			Marmalade
			Canned fruit
Meat	Fresh/frozen meat	Poultry	Poultry
		Other fresh meat	Unspecified fresh meat
	Meat products	Meat spread	Liver spread
			Other meat spread
		Other meat products	Other meat products
Fish	Fatty fish		Salmon
			Trout
			Other fatty fish
	Fish products	Fish spread	Fish spread
		Other fish product	Other fish product
	Shellfish		Shellfish
Eggs	Eggs		Eggs
Dairy	Milk	Whole milk	Whole milk
		Low-fat milk	Low-fat milk
			Skimmed milk
		Other milk	Unspecified milk
	Cheese	White cheese	White cheese, full-fat

			White cheese, semi-fat
		Brown cheese	Brown cheese
	Other dairy	Yoghurt	Yoghurt
		Cream	Cream
			Sour cream
		Ice cream	Ice cream
		Other dairy products	Other dairy products
Fats	Margarine		Soya margarine
			Margarine mixture
			Other margarine
			Light margarine
	Butter		Butter
			Butter-margarine mixture
			Unspecified butter
	Other fats		Mayonnaise
			Remoulade
			Dressing
			Mayonnaise in salads
Other	Sugar and sweets	Sugar	Sugar
			Other sweeteners
			Energy-free sweeteners
		Sweet spread	Sweat spread
			Honey
		Sweets	Chocolate
			Candy
	Snacks	Chips	Potato chips
		Nuts and seeds	Nuts and seeds
		Other snacks	Other snacks
Drinks	Coffee		Coffee
	Tea		Tea
	Soda	Soda sugar	Soda with sugar

		Lemonade with sugar
	Soda light	Soda light
		Lemonade light
Alcohol	Beer	Beer
		Alcohol-free beer
	Wine	Wine
	Liquor	Liquor

Supplementary Table 2a: Primary food subcategories contributing to the total intake of free choline.

Rank	Food item	Contribution, %	Cumulative contribution, %
1	Coffee	12.1	12.1
2	Potatoes	12.0	24.1
3	Low-fat milk	8.7	32.8
4	Wholegrain bread	8.3	41.1
5	Leafy vegetables	8.2	49.3
6	Fresh fruit	6.8	56.1
7	Alcohol	5.2	61.3
8	White bread	4.5	65.8
9	Other vegetables	3.8	69.6
10	Fish products	2.9	72.5

Supplementary Table 2b: Primary food subcategories contributing to the total intake of glycerophosphocholine.

Rank	Food item	Contribution, %	Cumulative contribution, %
1	Low-fat milk	31.7	31.7
2	Fish products	7.0	38.7
3	Fatty fish	5.5	44.2
4	Potatoes	5.4	49.6
5	Coffee	5.0	54.6
6	Fresh fruit	4.9	59.5
7	Fish spread	4.7	64.2
8	Alcohol	3.9	68.1
9	Yoghurt	3.7	71.8
10	Whole milk	3.6	75.4

Supplementary Table 2c: Primary food subcategories contributing to the total intake of phosphocholine.

Rank	Food item	Contribution, %	Cumulative contribution, %
1	Low-fat milk	26.7	26.7
2	Leafy vegetables	15.1	41.8
3	Potatoes	8.6	50.4
4	Other vegetables	6.3	56.7
5	Whole milk	4.2	60.9
6	Fresh fruit	4.0	64.9
7	Yoghurt	3.7	68.6
8	Other milk	2.8	71.4
9	Fish products	2.5	73.9
10	Poultry	2.1	76.0

Supplementary Table 2d: Primary food subcategories contributing to the total intake of phosphatidylcholine.

Rank	Food item	Contribution, %	Cumulative contribution, %
1	Eggs	33.8	33.8
2	Meat spread	6.3	40.1
3	Leafy vegetables	6.2	46.3
4	Pastries	4.9	51.2
5	Fish products	4.7	55.9
6	Wholegrain bread	4.7	60.6
7	Other meat products	4.4	65.0
8	Potatoes	4.2	69.2
9	Fish spread	3.8	73.0
10	Buns	3.8	76.8

Supplementary Table 2e: Primary food subcategories contributing to the total intake of sphingomyelin.

Rank	Food item	Contribution, %	Cumulative contribution, %
1	Eggs	15.1	15.1
2	Low-fat milk	12.2	27.3
3	Poultry	9.7	37.0
4	Fish products	7.8	44.8
5	Fish spread	6.4	51.2
6	White cheese	6.4	57.6
7	Wholegrain bread	6.4	64.0
8	Meat spread	6.1	70.1
9	Other meat products	5.4	75.5
10	Brown cheese	3.5	79.0

Supplementary Table 3: Overview of the contribution of food items to dietary intake (%) of total choline and individual choline forms.

Food items (%)	Total choline	Water-soluble forms			Lipid-soluble forms	
		Free choline	Glycerophospho-choline	Phosphocholine	Phosphatidyl-choline	Sphingomyelin
Dairy	19.20	15.01	46.05	40.46	4.36	30.43
Milk	14.52	11.12	38.56	33.72	2.06	15.20
Low-fat milk	11.83	8.69	31.74	26.72	1.72	12.23
Whole milk	1.46	1.49	3.56	4.23	0.14	1.66
Other milk	1.23	0.94	3.26	2.77	0.20	1.31
Cheese	1.88	1.82	1.16	1.32	1.48	9.94
White cheese	1.28	1.17	1.07	1.32	0.93	6.42
Brown cheese	0.60	0.65	0.09	0.00	0.54	3.52
Other dairy	2.80	2.05	6.34	5.42	0.81	5.34
Yoghurt	1.53	0.97	3.72	3.70	0.42	2.61
Cream	0.51	0.39	1.02	0.60	0.18	1.38
Ice-cream	0.58	0.40	1.30	0.81	0.18	1.34
Other dairy products	0.18	0.29	0.30	0.30	0.03	0.00

Drinks	6.97	18.73	9.45	0.00	0.06	0.00
Coffee	4.26	12.13	5.03	0.00	0.00	0.00
Tea	0.34	1.31	0.00	0.00	0.00	0.00
Soda	0.15	0.07	0.49	0.00	0.06	0.00
Alcohol	2.22	5.23	3.90	0.00	0.00	0.00
Eggs	15.31	0.15	0.17	0.80	33.75	15.10
Fats	1.49	0.16	0.57	0.95	2.75	1.69
Margarine	0.64	0.07	0.23	0.31	1.28	0.00
Butter	0.18	0.02	0.06	0.24	0.24	0.93
Other fats	0.62	0.05	0.25	0.07	1.16	0.75
Fish	8.55	4.62	13.00	3.27	8.65	13.15
Fatty fish	2.06	1.65	5.53	0.35	0.76	2.27
Fish products	4.82	2.86	7.01	2.49	4.74	7.75
Fish spread	3.54	1.77	4.73	1.54	3.84	6.44
Shellfish	1.67	0.12	0.46	0.33	3.16	3.13
Fruit	6.49	9.33	9.17	6.73	3.96	0.61

Fresh fruit	4.21	6.83	4.87	3.99	2.66	0.23
Juice	1.33	1.73	1.78	1.61	1.00	0.00
Other fruit	0.95	0.77	2.52	1.13	0.31	0.28
Grain products	12.84	16.54	7.56	5.54	14.42	13.98
Bread	8.49	13.37	5.97	3.31	7.70	9.73
White bread	2.82	4.49	1.96	1.34	2.54	2.85
Wholegrain bread	5.15	8.25	3.31	1.71	4.65	6.35
Other bread	0.53	0.64	0.69	0.24	0.50	0.48
Pastries	2.75	1.16	0.87	0.91	4.93	2.37
Buns	2.09	0.77	0.67	0.68	3.82	1.79
Biscuits	0.10	0.22	0.04	0.04	0.07	0.00
Other pastries	0.56	0.17	0.16	0.10	1.04	0.55
Other grains	1.60	2.01	0.71	1.32	1.79	1.88
Pizza	0.77	1.20	0.30	0.94	0.67	1.14
Cereal	0.83	0.81	0.41	0.37	1.13	0.74
Meat	10.23	3.61	3.68	4.94	16.52	23.75

Fresh meat	3.68	0.73	1.39	2.44	5.81	12.34
Poultry	2.24	0.61	0.14	2.14	3.51	9.68
Other fresh meat	1.44	0.11	1.25	0.16	2.30	2.70
Meat products	6.55	2.87	2.30	2.45	10.71	11.48
Meat spread	3.71	1.10	1.59	0.95	6.28	6.10
Other meat products	2.85	1.77	0.71	1.49	4.43	5.38
Other	0.52	0.68	0.10	0.23	0.72	0.00
Sugar and sweets	0	0	0	0	0	0
Snacks	0.52	0.68	0.10	0.23	0.72	0.00
Vegetables	17.22	30.20	7.78	35.64	14.18	0.23
Potatoes	6.33	12.05	5.36	8.59	4.21	0.00
Fresh vegetables	10.89	18.15	2.43	27.04	9.97	0.23
Root vegetables	1.99	5.38	0.76	5.23	0.32	0.00
Leafy vegetables	5.73	8.21	0.91	15.09	6.23	0.00
Other vegetables	2.57	3.78	0.59	6.27	2.65	0.00
Canned vegetables	0.60	0.82	0.13	0.41	0.76	0.23