Lean fish consumption is associated with decreased risk of metabolic syndrome

Results from a large population-based study

Christine Tørris

Dissertation for the Degree of PhD
Faculty of Medicine

UNIVERSITY OF OSLO

2017

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8377-058-2

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

Contents

Acknowledgements

As I come to the close of this long journey, the time has come to thank those who supported and helped me throughout this period. It has been a period of intense learning, not only in the scientific arena but also on a personal level.

First, I would like to express my sincere gratitude to my supervisors. To my main supervisor Milada Cvancarova Småstuen, I thank you for your excellent and efficient supervision, continuous support, motivation, and immense knowledge. Your technical and statistical skills are invaluable. To my co-supervisor Marianne Molin, I thank you for your critical reading and your sincere interest in this project. Your feedback was prompt, concise and thorough. Thank you both for your constructive guidance and encouragement along the way. You provided me with the tools that I needed to successfully complete my thesis.

I would also like to use this opportunity to thank the participants in the Tromsø study for your contribution, which is crucial for us to be able to do research.

I like to thank my friends and colleagues from Akershus University College of Applied Sciences, for your support throughout this work.

Finally, I thank my family for your support and inspiration throughout this work and for always believing in me. My deepest gratitude goes to my parents for your wise counsel and sympathetic ear. You are always there for me. To my children and husband, for your support and for helping me recharge my batteries and balance my perspectives, and to my friends, who were happily talking about other things than my PhD work.

Christine

Oslo, March 2017

Abbreviations

ATP III	Adult Treatment Panel III
BMI	Body mass index
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DHA	Docosahexaenoic acid
DM2	Diabetes mellitus type 2
EPA	Eicosapentaenoic acid
FFA	Free fatty acids
FFQ	Food frequency questionnaires
HDL	High-density lipoprotein
IDF	International Diabetes Federation
IL-6	Interleukin-6
JIS	Joint Interim Societies
LDL	Low-density lipoprotein
LOQ	Limit of quantification
MetS	Metabolic syndrome
MUFA	Monounsaturated fatty acids
PCB	Polychlorinated biphenyl

POP	Persistent organic pollutant
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PUFA	Polyunsaturated fatty acids
SAD	Sagittal abdominal diameter
SBP	Systolic blood pressure
SFA	Saturated fatty acids
TG	Triglycerides
TNF-α	Tumor necrosis factor alpha
VLDL	Very-low-density lipoprotein
WC	Waist circumference
WHO	World Health Organization

Summary

Background

Metabolic syndrome (MetS) is a cluster of metabolic abnormalities including abdominal obesity, dyslipidemia, hyperglycemia and hypertension. The negative impact of MetS on public health is profound. A lifestyle factor such as fish intake may be a useful component in a dietary strategy aiming to improve components of MetS. Few studies have investigated associations between fish consumption and MetS and the published results are not conclusive.

The aim of this study was to identify associations between fish consumption and MetS and its components as well as to distinguish the associations of fatty or lean fish consumption. The overall hypothesis was that higher fish consumption is associated with a lower prevalence of MetS and a healthier metabolic profile.

Methods

Current literature was reviewed before investigating possible associations between fish consumption and MetS. The prevalence of MetS and its associations with fish consumption were investigated at two different time points using data from the fourth survey of the Tromsø Study (Tromsø 4) carried out in 1994–95 (n=27,158) and the sixth survey of the Tromsø Study (Tromsø 6) carried out in 2007–2008 (n=12,984). Furthermore, possible associations between fish consumption and changes in MetS components during the 13-year follow-up period were investigated (from Tromsø 4 to Tromsø 6). MetS was defined using the definition of the Joint Interim Societies (JIS), in which one point is given for each fulfilled MetS criteria (metabolic score). Dietary data were collected via food frequency questionnaires (FFQ) (Tromsø 4). Non-fasting blood samples were taken, and all measurements were carried out according to standard protocols. The Regional Committee for Medical Research Ethics, South East Norway, approved the study.

Results

Current literature on the topic was reviewed (Paper I); scarce information regarding associations between fish consumption and MetS was found. Only seven studies were identified for inclusion in the review after identifying 502 literature citations in PubMed. Four studies (one follow-up and three cross-sectional) found associations between fish consumption and MetS (three among men and one among women).

In Tromsø 4 (1994–1995), the prevalence of MetS was 8.1% in the whole sample. Fish consumption once a week or more (fatty and lean fish for dinner) was associated with a 36% lower risk of having MetS compared to those consuming fish less than once a week among those aged 60–70 years. Lean fish was associated with a 35% lower risk of having MetS compared to those consuming lean fish less than once a week in the same age group. No association was found for fatty fish consumption (Paper II). In Tromsø 6 (2007-2008), the prevalence of MetS was 22.6% in the whole sample. Fish consumption once a week or more was associated with a lower risk of having MetS among men (OR 0.85, CI 95% 0.74 to 0.98). In adjusted models, lean fish consumption was associated with a decreased risk of having MetS, whereas fatty fish consumption was not associated with decreased risk (Paper III). Higher fish consumption was associated with a healthier lipid profile with increased high-density lipoprotein (HDL) cholesterol and decreased triglycerides; this was found both in Tromsø 4 and in Tromsø 6 (Papers II and III).

During the 13-year follow-up period (Paper IV), lean fish consumption once a week or more was significantly associated with decreased future metabolic score, decreased triglycerides and increased HDL-cholesterol, whereas decreased waist circumference and blood pressure were identified only for men (age-adjusted models). Fatty fish consumption was significantly associated with increased waist circumference for both genders and increased HDL-cholesterol levels in men (age-adjusted model).

Conclusions

These findings support the hypothesis that fish consumption may be associated with a lower risk of MetS and a healthier metabolic profile. Furthermore, lean fish consumption may have a role in MetS prevention. However, age and gender also seem to influence the results. These results should be regarded as a basis for generating hypotheses and should be further tested in other studies, especially to clarify the role of lean versus fatty fish consumption with regard to MetS risk.

LIST OF PAPERS

This thesis is based on the following papers, which are referred to in the text by the Roman numerals I–IV:

Paper I

Tørris C, Molin M, Cvancarova Småstuen M. *Fish consumption and its possible preventive role on the development and prevalence of metabolic syndrome - a systematic review*. Diabetology & Metabolic Syndrome 2014; 6:112.

Paper II

Tørris C, Molin M, Cvancarova Småstuen M. *Associations between fish consumption and metabolic syndrome. A large cross-sectional study from the Norwegian Tromsø Study: Tromsø 4*. Diabetology & Metabolic Syndrome 2016; 8:18.

Paper III

Tørris C, Molin M, Cvancarova Småstuen M. *Lean fish consumption is associated with lower risk of metabolic syndrome: a Norwegian cross sectional study*. BMC Public Health 2016; 16:347.

Paper IV

Tørris C, Molin M, Cvancarova Småstuen M. *Lean Fish Consumption Is Associated with Beneficial Changes in the Metabolic Syndrome Components: A 13-Year Follow-Up Study from the Norwegian Tromsø Study.* Nutrients 2017; 9:247.

1 Background

Metabolic syndrome (MetS) consists of different risk factors for cardiovascular disease (CVD) and diabetes mellitus type 2 (DM2), that occur together more often than by chance alone [1]. MetS involves abdominal obesity, insulin resistance and chronic inflammation [2] and has been associated with higher serum C-reactive protein (CRP) [3], which is a systemic inflammation marker.

Today, lifestyle interventions remain the primary strategy in MetS therapy, and lifestyle factors such as diet may be useful in reducing the prevalence of MetS. It is therefore of great importance to identify strategies that contribute to preventing and reversing the syndrome together with its components. The benefits of fish consumption on cardiovascular disease (CVD) have been described previously [4-6], and an inverse relationship has been identified between consumption of fish and heart failure [7], cerebrovascular disease [8], ischemic stroke [9] and sudden coronary death risk [10]. Particularly, fatty fish consumption has been suggested to reduce the risk of CVD due to its high level of n-3 fatty acids [4]. Fish consumption has also been associated with reduced levels of several CVD risk markers such as triglycerides, systolic blood pressure and fasting glucose levels [11]. Increased consumption of fish has also been associated with a lower risk of DM2 [12], especially consumption of lean fish [13]. Furthermore, fish consumption has also been associated with lower serum CRP [14].

Some studies have also suggested an inverse association between fish consumption and MetS among men [15-17]. However, most studies focused on single components in marine species, such as n-3 fatty acids [18, 19]. It seems that there is a lack of evidence focusing on the consumption of fish by humans and its possible health effects related to MetS, in particular regarding distinguishing between the effects of lean and fatty fish consumption. We therefore wanted to examine associations between consumption of fish, distinguishing lean and fatty fish, and health effects related to MetS and its components.

1.1 Metabolic syndrome

1.1.1 Definition of metabolic syndrome

MetS consists of a cluster of metabolic abnormalities including abdominal obesity, dyslipidemia, hyperglycemia and hypertension [1]. This constellation of risk factors for cardiovascular disease was described in the 1920s by Kylin as the clustering of hypertension, hyperglycemia and gout [20]. Later, in 1947, attention was drawn to upper body adiposity by Vague [20]. Different concepts of MetS have existed for almost a century, with names like syndrome X, insulin resistance syndrome and the deadly quartet [20]. In 1998, a consulting group for the World Health Organization (WHO) formed a definition wherein insulin resistance (IR) was emphasized as the major underlying risk factor, and therefore required in the diagnosis of MetS [21]. In 2001, the National Cholesterol Education Program Adult Treatment Panel III (ATP III) presented a different set of criteria, wherein the presence of any three of five factors established the diagnosis (abdominal obesity, elevated triglycerides, reduced high-density lipoprotein (HDL) cholesterol, elevated blood pressure and elevated fasting glucose) [22]. In 2005, The International Diabetes Federation (IDF) dropped the WHO requirement for insulin resistance and made abdominal obesity necessary among the five factors required in the diagnosis [1].

Then, after several attempts to unify criteria for MetS, a clinical definition was agreed upon through a Joint Interim Statement (JIS) in 2009 [1]. In this harmonized definition, the presence of any three of five listed risk factors (abdominal obesity, elevated triglycerides, reduced HDL cholesterol, elevated blood pressure and elevated fasting glucose) constitutes a diagnosis of MetS [1]. Waist circumference is no longer an obligatory component, but it remains a preliminary screening tool. In the JIS definition, special criteria are used for all of the MetS components, except waist circumference, for which national or regional cut-off points for waist circumference are recommended. This is due to the complexity of defining abdominal obesity in different populations and ethnic groups [1]. In Norway, abdominal obesity is defined according to the IDF criteria, with the cut-off at waist circumference ≥94 cm among men and ≥80 cm among women [23] (**Table 1**).

Abdominal obesity can be measured using waist circumference or sagittal abdominal diameter, however (**Figure 1**). Waist circumference is measured at the level of the belly button or at the midpoint between the lower border of the rib cage and the iliac crest if the belly button is pointing downwards in obese individuals [24, 25].

Table 1. Criteria for defining metabolic syndrome.

Criteria	Definition	Men	Women
WC	IDF	≥94 cm	≥80 cm
	ATP III	>102 cm	>88 cm
S-HDL-C	ATP III, IDF, JIS	<1.0 mmol/L (40 mg/dL)	<1.3 mmol/L (50 mg/dL)
S-TG	ATP III, IDF, JIS	>1.7 mmol/L (150 mg/dL)	
Blood pressure	ATP III, IDF, JIS	SBP ≥130 mm Hg	
		DBP ≥85 mm Hg	
Fasting S-glucose	IDF, JIS	≥5.6 mmol/L (100 mg/dL)	

Adapted from Alberti et al. 2009 [1].
ATP III: Adult Treatment Panel III; three criteria must be fulfilled to diagnose MetS.
IDF: International Diabetes Foundation; the criterion of waist (population specific) and two of the other criteria must be fulfilled to diagnose MetS.
JIS: Joint Interim Statement, the criterion of waist (population specific) and two of the other criteria must be fulfilled to diagnose MetS.
WC: waist circumference, S-TG: S-triglycerides, S-HDL-C: high-density lipoprotein cholesterol, SBP: systolic blood pressure, DBP: diastolic blood pressure.

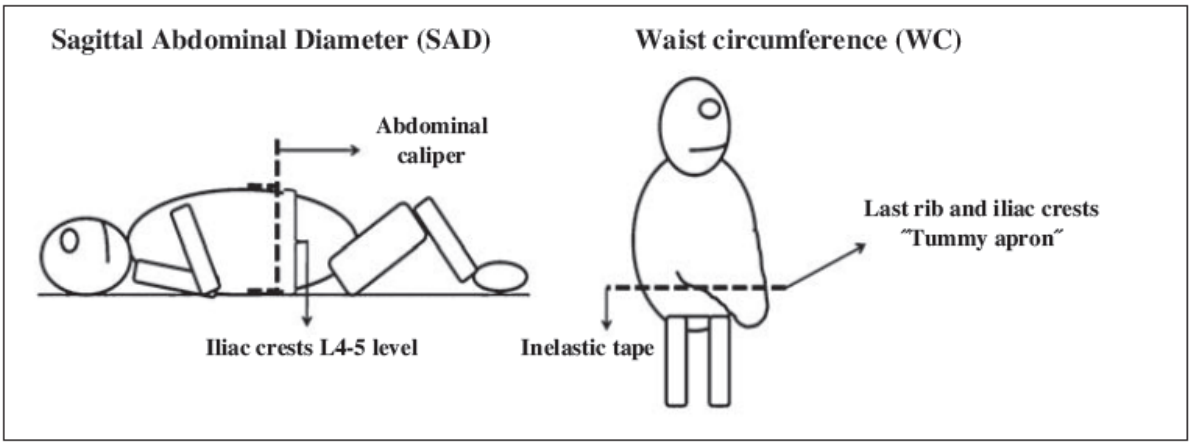

Figure 1. Measurement of abdominal obesity [24].

1.1.2 Prevalence of metabolic syndrome

Comparing the prevalence of MetS is a challenge, due to the fact that different definitions of MetS are used in different studies. However, studies from most countries indicate that 20–30% of the adult population can be characterized as having MetS, with prevalence increasing with age [26].

In addition to age, in women, both parity and increased numbers of children have been associated with higher rates of MetS [27]. However, some studies have suggested a decreased risk of MetS in women with a history of breastfeeding [27]; in particular, longer duration of lactation has been associated with lower incidence of MetS even years after weaning [28]. Earlier findings have suggested an association between prolonged lactation and a healthier metabolic profile and body composition, especially with regard to lipid levels and waist-to-hip ratio, several years after weaning [29].

The prevalence of MetS also depends on the sample population studied (e.g., gender, age, race and ethnicity) as well as lifestyle and socioeconomic status (SES) [30]. However, as obesity is a driver of MetS, the highest prevalence of MetS is found in obese populations [26]. When comparing gender differences in the prevalence of MetS some studies have observed increased prevalence among men [16, 31-33] and some among women [34, 35], and some studies have found no gender-related differences [36-38].

In line with what has been observed in other countries in the developed world, a prevalence between 17% and 46% has been observed in Europe [26]. In Norway, similar prevalence of MetS has been found among participants from the Nord-Trøndelag Health Study 1995–1997 (HUNT 2) [39]. However, when using the IDF definition, MetS prevalence was slightly higher (29.6%, 95% CI 28.8 to 30.5) compared to the 2005 ATP III criteria (25.9%, 95% CI 25.0 to 26.7). When comparing men and women, the prevalence of MetS was slightly higher among men aged <70 years compared to prevalence among women (**Figures 2 and 3**) [39].

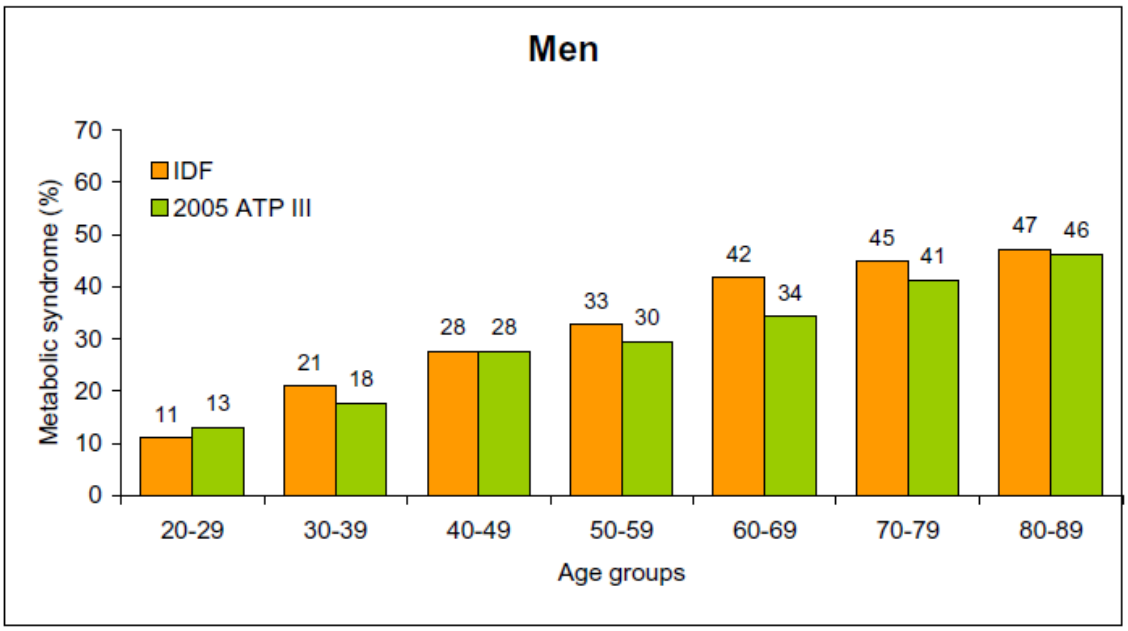

Figure 2. Age-specific prevalence of metabolic syndrome in Norway among men (HUNT 2) [39].

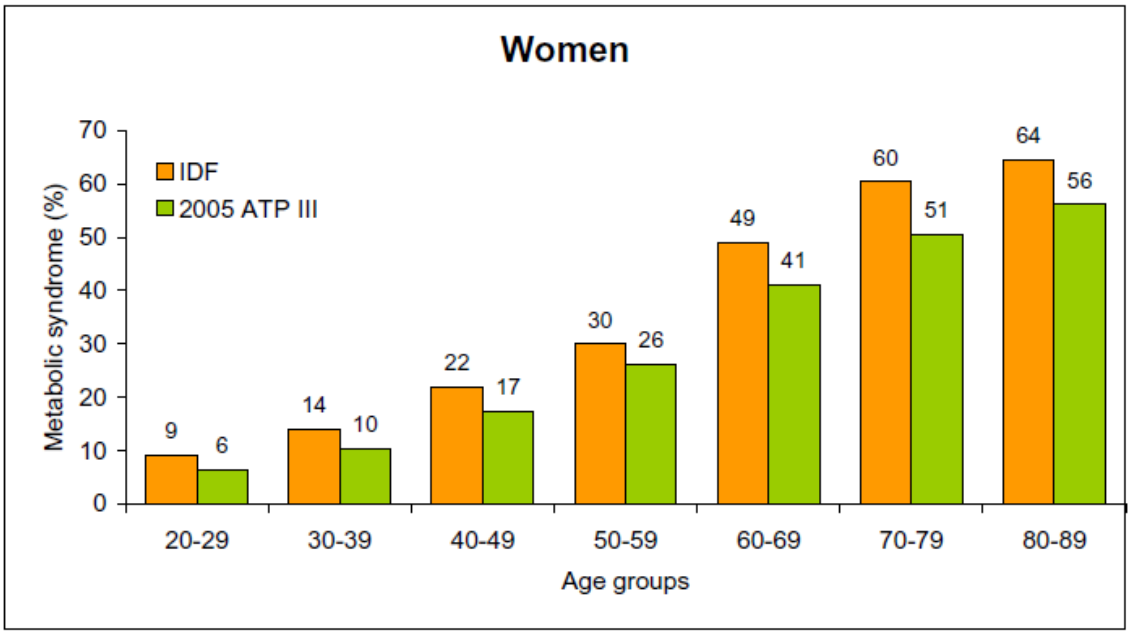

Figure 3. Age-specific prevalence of metabolic syndrome in Norway among women (HUNT 2) [39].

1.1.3 Pathophysiology of metabolic syndrome

Together with different definitions of MetS, different pathophysiological explanations for MetS have been proposed over the years. The pathophysiology of MetS is complex and involves insulin resistance, chronic inflammation and ectopic fat accumulation followed by adipose tissue saturation [2]. However, abdominal obesity appears to precede the appearance of the other MetS components, that is, dyslipidemia, hypertension and hyperglycemia [40].

The initiation of MetS is influenced by genetic predisposition and environmental factors, such as a sedentary lifestyle together with a diet containing excess calories [2, 41]. This leads to obesity, with increased storage of fat in the adipose tissue as a reflection of the positive energy balance (more in than out). Adipose tissue is a major energy storage organ, consisting of lipids, adipocytes, fibroblasts, endothelial cells, macrophages and preadipocytes [42]. However, adipose tissue not only stores but also releases fatty acids.

When the adipocytes' ability to store fat (triglycerides) is exceeded, fatty acids are released from the adipose tissue, resulting in an elevation of circulating free fatty acids (FFA) [20, 41] (**Figure 4**). The increased level of FFA reduces insulin sensitivity in muscle and other tissue by inhibiting the insulin-mediated glucose uptake. This results in an increase in circulating glucose together with increased pancreatic insulin secretion, resulting in hyperinsulinemia, wherein excess levels of insulin circulate in the blood relative to the level of glucose. This increase of FFA and insulin in the blood also contributes to hypertension mechanisms such as enhanced sodium reabsorption [20]. Furthermore, insulin action also has a role in controlling sympathetic nervous system activity, which increases in response to raised insulin levels; however, in obesity this over-activity may cause hypertension [41].

The expansion of adipose tissue due to adipocyte hypertrophy also leads to an inflammatory response in the fat tissue due to the infiltration of macrophages and other immune cells, which release pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) [20, 41]. TNF-α influences the immune system by increasing the permeability and adhesiveness of the blood vessels, while IL-6 stimulates hepatocytes to synthesize CRP [43].

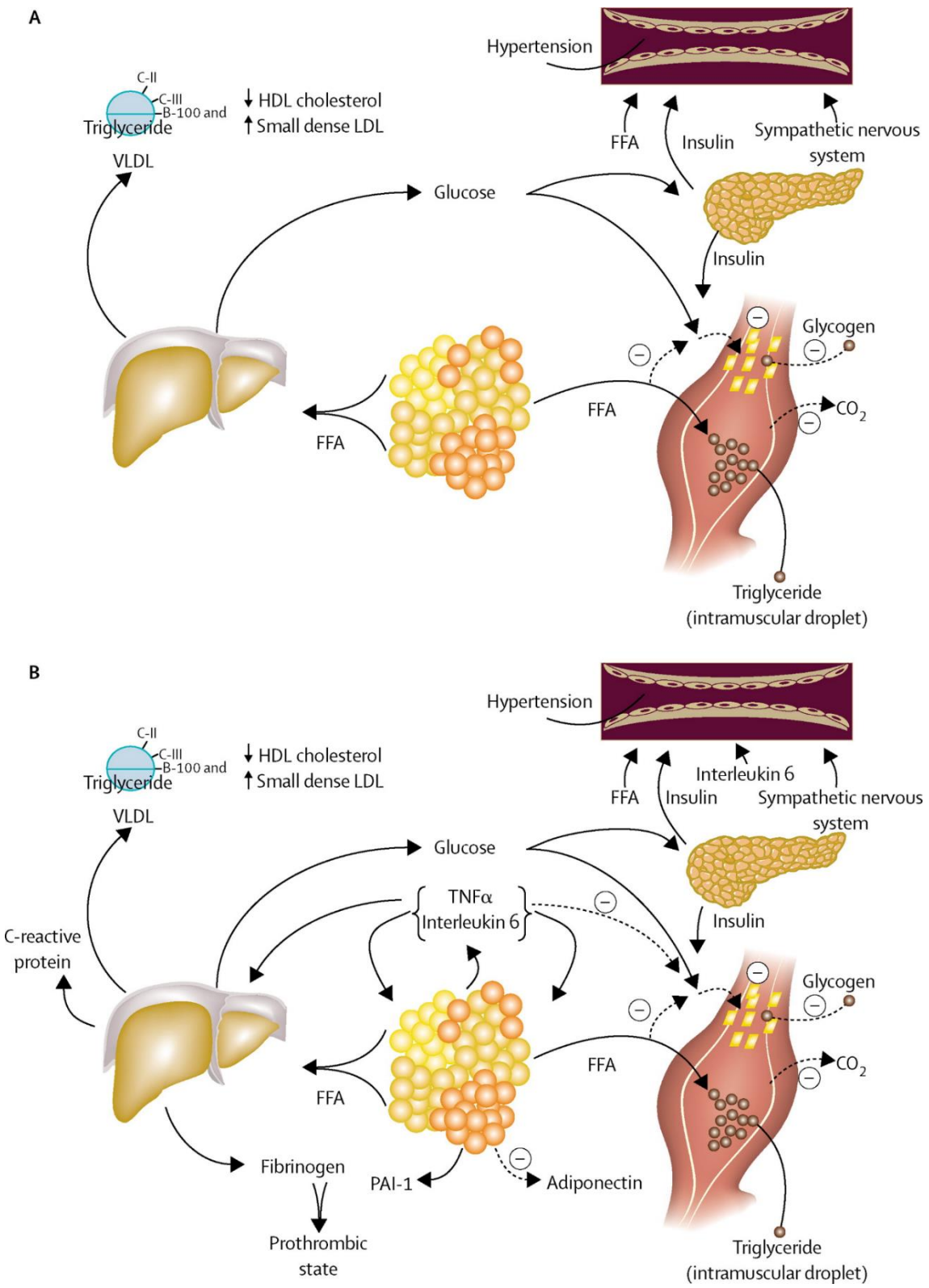

Figure 4. Pathophysiology of metabolic syndrome. Reproduced with permission from Elsevier [20]. A: Free fatty acids (FFA) release from adipose tissue, leading to increased production of glucose and triglycerides from the liver and reduced insulin sensitivity in muscle. Hyperinsulinemia and increased sympathetic nervous system (SNS) activity contributes to hypertension. B: An inflammatory response in the fat tissue release pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and plasminogen activator inhibitor 1 (PAI-1).

1.2 Fish consumption

1.2.1 Fish consumption in Norway

Fishing has always been important in Norway. Its long coastline is especially suitable for fishing and fishing has traditionally provided an important nutritional resource. Today, consumption of meat is dominant and is higher than fish consumption in Norway [44]. According to the Norwegian dietary recommendations, a healthy diet should include at least 2–3 portions or 300–450 grams of fish weekly, including a minimum of 200 grams of fatty fish [45].

In the national dietary survey Norkost 1997, the average fish consumption was 58 grams per day among women, and 78 grams per day among men [46]. The consumption of fish was 30% higher in Northern Norway compared with the average for the country [46]. In Norkost 3 (2010–2011), the average fish consumption was 56 grams per day among women and 79 grams per day among men [47]. In both surveys, fish consumption increased with age, and men were found to consume more fish compared to women [46, 47]. In Norkost 1997, those with at least 13 years of education consumed slightly more fish compared to those with less education (women 5 g/day and men 7 g/d) [46], while Norkost 3 (2010–2011) did not observe any difference in education among those with high and low fish consumption [47].

Fish consumption increased slightly from 2004 to 2013, although preliminary estimates suggest a decline in fish consumption in 2014 [44]. However, self-caught fish is not included, which might have led to some underestimation and possible bias. Regardless, the main aim of our study was not to estimate overall consumption but to compare consumption groups, and we find it reasonable to assume that the proportion of self-caught fish will be similar in the compared groups. The proportion of participants who reported that they consumed fish for dinner at least three times per week increased from 22% to 25% from 2005 to 2013. A lower percentage was observed among those with less education compared to those with more education; however, increased fish consumption was observed in both educational groups (e.g., less/more education) [44].

1.2.2 Nutrients in fish

Fish is an important source of a variety of nutrients [48] (**Table 2**), that may contribute to a healthier metabolic profile [49, 50].

Table 2 Nutritional profile of commonly consumed, whole, raw fish, per 100 g [51, 52].

Nutrients	**Cod**		**Salmon**	
	Wild	Farmed	Wild	Farmed
Energy (kJ)	343	358	760	932
Fat (g)	1.1	0.5	12	16
SFA (g) [1]	0.1	0.1	1.8	3
MUFA (g) [1]	0.1	0	4.4	5.9
PUFA (g) [1]	0.3	0.2	1.9	5
Omega-3 (g)	0.5	0.2	1	1.5
Protein (g)	17.9	20	19.7	20
Taurine (mg)	108	-[2]	60	60
Fat-soluble vitamins				
Retinol (µg)	12	2	0	26
Vitamin D (µg)	2	0.7	8	10
Water soluble vitamins				
Niacin (mg)	1.8	3.9	7	7.3
Vitamin B6 (mg)	0.12	0.26	0.6	0.51
Folate (µg)	11	11	13	7
Vitamin B12 (µg)	1.5	1	6.9	3.5
Minerals and trace elements				
Selenium (µg)	22	30	50	30
Iodine (µg)	119	300	-[2]	10

[1] SFA: saturated fatty acids, MUFA: monounsaturated fatty acids and PUFA: polyunsaturated fatty acids. [2] No information.

Fish can be classified as lean, medium-fatty or fatty depending on the amount of fat in the body tissue [53] (**Table 3**). However, it is common to classify fish as lean or fatty, with both medium-fatty and fatty fish classified as fatty fish [51].

Whereas the amount of fat is relatively stable in lean fish, the amount may vary considerably in fatty fish. Lean fish such as cod contains less than 2 g of fat per 100 g, while fatty fish contains more than 8 g of fat per 100 g [53]. The marine n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are present mainly in fatty fish, such as salmon and mackerel; however, lean fish also contains n-3 fatty acids. Along with n-3 fatty acids, fish is a

good source of nutrients such as well-balanced proteins, selenium, iodine, vitamin D, choline and taurine that may also contribute to the health benefits of fish consumption [48]. While fatty fish is considered a valuable source of n-3 fatty acids and vitamin D, lean fish has a much lower level of total energy and total lipids compared to fatty fish. Furthermore, lean fish contains more iodine compared to fatty fish [48].

There are also differences between wild and farmed fish depending on their feed, and the nutrients in farmed fish reflect the composition of fish feed [48]. Both farmed fatty fish and farmed lean fish (cod) contains more energy compared to wild fish. Farmed fatty fish has a higher level of total lipids compared to wild fatty fish, and farmed fatty fish also contains more saturated fatty acids (SFA) and mono- and polyunsaturated fatty acids (MUFA and PUFA, respectively).

Table 3. Fat content per 100 g in different species [53].

Fat content per 100 g	Lean fish <2 g	Medium-fatty fish 2–8 g	Fatty fish[1] >8 g
Fish species	Cod	Catfish	Eel
	Haddock	Halibut	Herring
	Pike	Tuna	Salmon
	Plaice	Winter mackerel	Summer mackerel
	Saithe		Trout

[1] When fish is classified as lean or fatty, both medium-fatty and fatty fish are classified as fatty fish.

1.2.3 Contaminants and other undesired substances in fish

Many positive health effects of fish consumption have been pointed out over the last decades. However, fish may also contribute to dietary exposure to contaminants and other undesired substances. The main contaminants that may pose a potential risk to public health through fish consumption are considered to be methylmercury, dioxins and dioxin-like polychlorinated biphenyls (PCBs) [48]. Mercury is released into the environment, and is found in different chemical forms. The most common form of organic mercury in the food chain is methylmercury, which accumulates in the body and crosses the placenta- and blood–brain barriers [48]. Dioxins and PCBs comprise a subgroup among the persistent organic pollutants (POPs), consisting of fat-soluble chlorinated organic compounds that are found in the highest

concentrations high up in the food chain [48]. The major dietary exposure source of dioxins and PCBs is marine fat. In addition, medicine (e.g., antibiotics and agents against sea lice) also leaves residues in farmed fish [48]. Nevertheless, the benefits of fish consumption have been found to outweigh any negligible risk presented by contaminants and other known undesirable substances in fish [48].

2 Aims of this thesis

The overall aim of the thesis was as follows:

- To identify associations between fish consumption and MetS and its components.

Our overall hypothesis was that higher fish consumption is associated with a lower prevalence of MetS as well as a healthier metabolic profile.

The specific aims of the thesis were as follows:

- To review the current literature on fish consumption and the possible preventive role of on fish consumption in the development and prevalence of MetS (Paper I).
- To identify possible associations between fish consumption and MetS and its components at two different time points, including differences regarding consumption of fatty and lean fish (Papers II and III).
- To identify possible associations between fish consumption, both fatty and lean, and changes in MetS components during a 13-year follow-up period, including changes in metabolic score (Paper IV).

3 Materials and methods

3.1 Paper I

Paper I is a review article. To examine how fish consumption affects the development and prevalence of MetS, the paper reviews cross-sectional, prospective cohort and intervention studies conducted among adult humans. Studies reporting fish or seafood consumption related to MetS (prevalence or incidence) among adults were included. The literature search was performed in PubMed and was restricted to papers written in English using an established definition of MetS. Studies conducted among children and animal studies were excluded. The search terms used were "fish", "seafood", "intake" and "consumption" as exposure search terms and "metabolic syndrome" or "insulin resistance" as outcome search terms. The last search was performed June 1, 2014. However, the time span for the search was ten months. Citations were identified, and potential abstracts and full-text articles were screened. Full-text articles were assessed for eligibility, and seven studies were ultimately included in the review after exclusion of ineligible papers (**Figure 5**).

Prospective cohort studies were considered eligible for inclusion if they had at least one year of follow-up and involved general populations. Insulin resistance syndrome (IRS) was accepted as an outcome factor and considered for inclusion when it was defined using the same definition as in MetS. Abstracts, letters and reviews were not included but were inspected for additional references that might meet the inclusion criteria. Reference lists of the included studies and relevant published reviews were examined to identify additional papers for possible inclusion.

The full-text of the article was retrieved whenever there was uncertainty about a study's match with the inclusion criteria. The included studies were assessed according to the quality of the study design and methods, the measurements of MetS and fish consumption, and the statistical analysis. The review procedure was carried out in accordance with the PRISMA statement for review reporting [54], and a protocol was developed for study selection.

The following data were collected from the different studies included in the review: citation, country where the study was performed, design, aim, participants' age and gender (baseline age and duration of follow-up for prospective studies), sample size, methods of measurement,

variables adjusted for in the analysis, multivariate adjusted OR with a 95% CI for the lowest versus the highest consumption of fish and MetS incidence or prevalence.

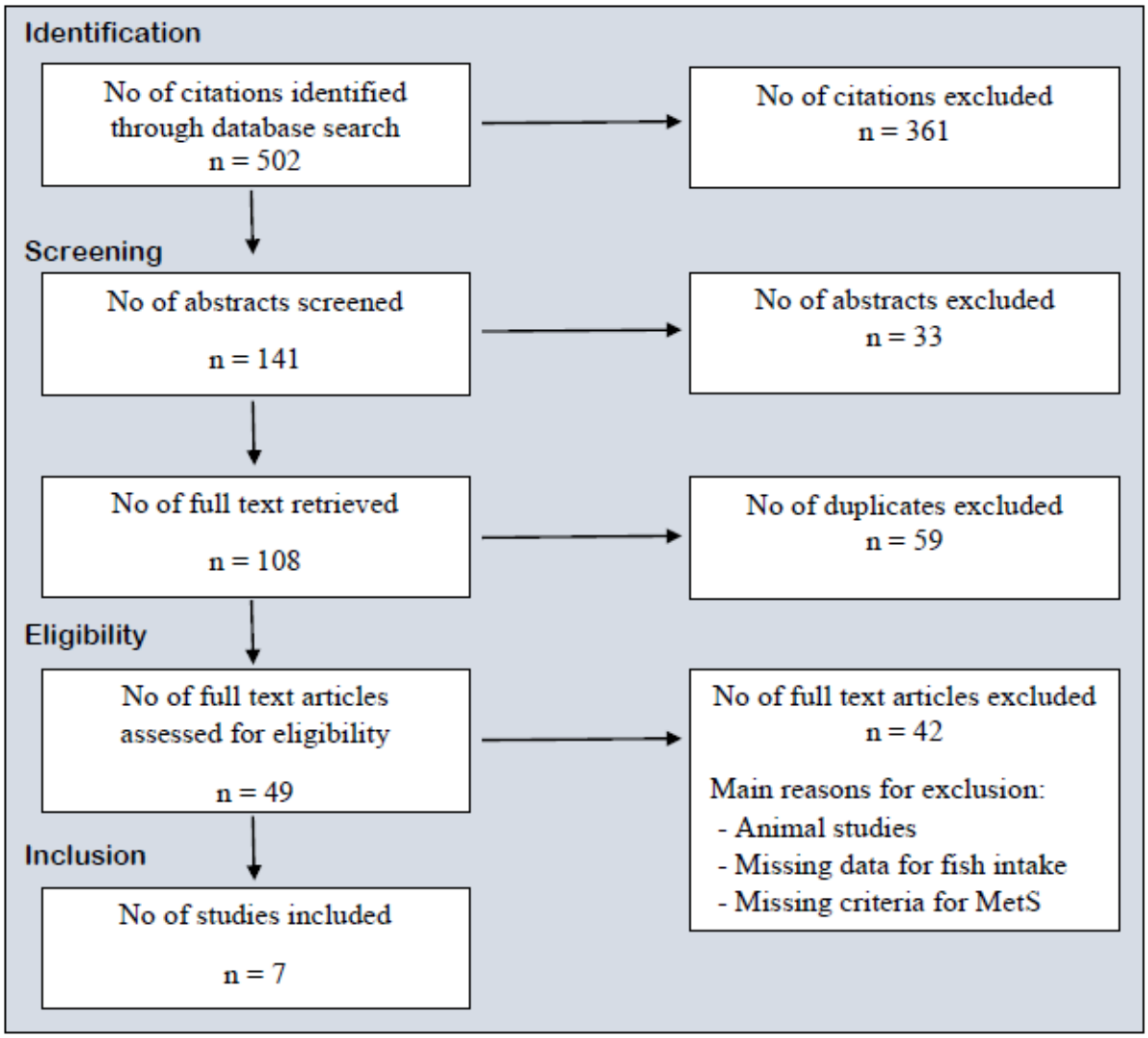

Figure 5. Flow of studies through the different phases of this systematic review [55].

3.2 Papers II–IV

3.2.1 The Tromsø study

The Tromsø study is a Norwegian population-based prospective study consisting of six surveys, referred to as Tromsø 1–6, wherein whole birth cohorts from the municipality of

Tromsø were invited to participate [56]. Papers II–IV are based on pre-collected data from two of the surveys in the Tromsø study, the fourth survey of the Tromsø study (Tromsø 4) in 1994–1995 and the sixth survey of the Tromsø study (Tromsø 6) in 2007–2008. Papers II and III have a cross-sectional design and are based on Tromsø 4 (Paper II) and Tromsø 6 (Paper III). Paper IV is a follow-up study using dietary data from the Tromsø 4 survey (1994–1995) and MetS data from both the Tromsø 4 survey (1994–1995), and the Tromsø 6 survey (2007–2008) were used to identify possible associations between fish consumption and changes in MetS components and metabolic score during a 13-year follow up period (Paper IV).

Table 4. The Tromsø study (Tromsø 1–6). Adapted from Eggen et al. [57].

Name of the survey	Year of the survey	Number of participants
Tromsø 1	1974	6,595
Tromsø 2	1979–1980	16,621
Tromsø 3	1986–1987	21,826
Tromsø 4	1994–1995	27,158
Tromsø 5	2001–2002	8,130
Tromsø 6	2007–2008	12,984

The Tromsø study was funded by the University of Tromsø, and received additional contributions directly or indirectly from others, such as the National Screening Services, the Research Council of Norway, Northern Norway Regional Health Authority, Norwegian Council on Cardiovascular Diseases and Norwegian Foundation for Health and Rehabilitation [56].

The Tromsø study was initiated in 1974 due to the high mortality from cardiovascular diseases (CVD) in Norway at that time [56] (**Table 4**). The Tromsø study was conducted in the municipality of Tromsø, where most of the inhabitants live in the urban areas around the city center. Participants who attended in the earlier rounds of this survey as well as a random sample drawn from the population of all individuals living in this area were invited to attend in the last surveys. The vast majority of the participants are Caucasians, mainly of Norwegian origin, with limited ethnic diversity. However, it also includes Sami and Finnish minorities [56, 57]. The attendance rates were >75% in Tromsø 1–5 and 66% in Tromsø 6; the latter is

due to a lower attendance rate among the relatively young and those who had never participated previously [56]. The subjects were free to attend whenever suitable within the time frame of one year, and non-attendees were given one reminder. Data was collected through questionnaires, serum samples and anthropometrical measurements. The participants answered two questionnaires. The first questionnaire was enclosed in the invitation letter and the second questionnaire was given to participants during their first visit. All the surveys, examinations, measurements and laboratory work followed standardized procedures and were performed by trained health personnel [56, 57].

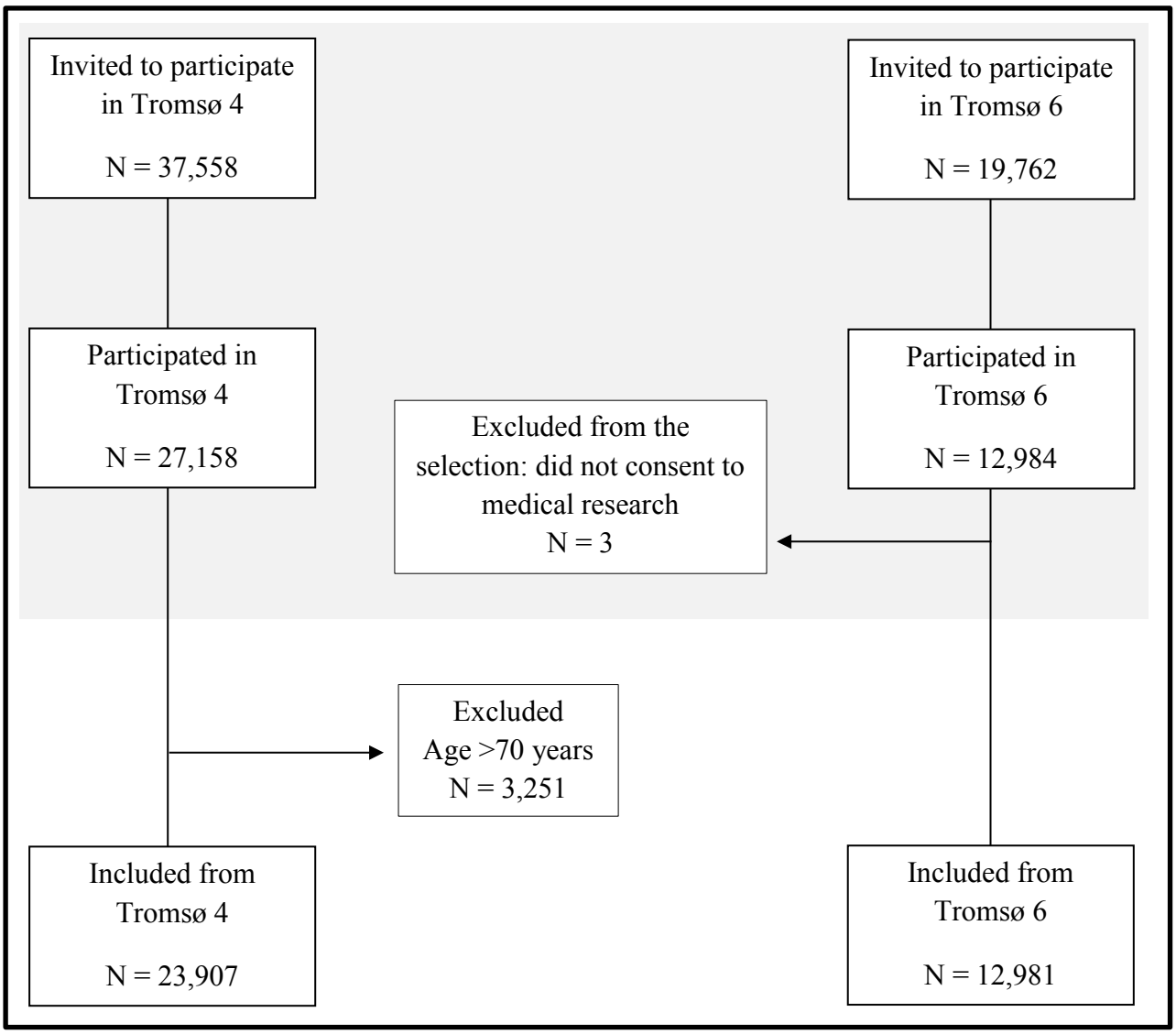

Figure 6. The study sample (Papers II–IV).

3.2.2 The Tromsø study: Tromsø 4

In Tromsø 4 (1994–1995), 37,558 men and women were invited to participate, and a total of 27,158 participated [56]. The majority of the sample population lived in the city of Tromsø [58]. The attendance rate was 70% among men and 75% among women [56]. Participants aged <70 years received a separate questionnaire which included questions on fish consumption (fatty/lean/processed); therefore only participants <70 years were included in the present study (Papers II and IV) (**Figure 6**).

Questionnaires and serum samples in Tromsø 4

A questionnaire was enclosed in the invitation letter. During the first visit, blood pressure and a blood sample for serum measurements were taken and participants were given a second questionnaire consisting of questions regarding dietary habits, dietary supplements and pregnancy (parity, lactation length in months per child) to be returned by mail after the first visit. The same questionnaire was given to both men and women, but a separate section contained questions for women only.

Fish consumption was assessed as weekly frequency in the questionnaire (never, <1, 1, 2–3, 4–5, approximately every day), including consumption of fatty fish such as salmon and lean fish such as cod. The computation of energy intake included both table fat, frequency of different foods and alcoholic beverages [58].

Three blood pressure readings were taken during the first visit. During the second visit, anthropometrical measurements (waist) and non-fasting serum samples were taken (triglycerides, HDL cholesterol, glucose).

3.2.3 The Tromsø study: Tromsø 6

Tromsø 6 was conducted in 2007–2008, and 12,984 participants attended (6,054 men and 6,930 women, aged 30–87) [56]. The attendance rate was 63% among men and 68% among women [56]. Based on the official population registry, the inhabitants of Tromsø were invited to attend, and a questionnaire was enclosed in the invitation. During the first visit, different

measurements (height, weight, waist, blood pressure) and a blood sample for serum measurements were taken (HDL cholesterol, triglycerides and glucose). The attending participants were then given a second questionnaire. The questionnaire was completed at home and brought to the study site, where it was checked for inconsistencies and incomplete data. Participants answered questions concerning demographic data such as age, education and physical activity in addition to health and diet [56, 57].

Fish consumption was assessed using a food frequency questionnaire (FFQ), regarding consumption of fatty fish (e.g., salmon, trout, mackerel, herring, halibut, redfish) and lean fish (0–1 times per month, 2–3 times per month, 1–3 times per week, 4–6 times per week, 1–2 times per day).

Waist circumference was measured across the belly button without outerwear, using a measuring tape [57]. Blood pressure was measured using an automated device (Dinamap Pro Care 300 Monitor, GE Healthcare, Norway), and the correct cuff size was selected after measuring the circumference of the upper right arm. Three readings were taken in a sitting position, separated by 1-min intervals and after a 2-min rest.

To collect blood samples for measurement of blood lipids and glucose, venipuncture was performed with participants in a sitting position. Participants were not required to fast but were allowed to drink only water and black coffee during their visits. A light tourniquet was used and released before sampling. The blood samples were sent to the Department of Laboratory Medicine, University Hospital North Norway, Tromsø (ISO-standard 17025) [57]. All examinations, measurements and laboratory work followed standardized procedures and was performed by trained health personnel [57]. From Tromsø 6, 12,981 participants were included in the present study (**Figure 6**).

3.2.4 Ethical considerations

The Tromsø Study was approved by the Data Inspectorate of Norway and the Regional Committee of Medical and Health Research Ethics, North Norway [57, 59]. Participation in the surveys was voluntary, and each subject gave written informed consent prior to participation [57, 59].

This PhD-project was approved by the Regional Committee for Medical Research Ethics, South East Norway.

3.3 Definitions used in statistical analyses

3.3.1 Metabolic syndrome definition

Different criteria have been used to define MetS over the years, both in Norway and in other parts of the world. However, today the recommended definition for the European population is the JIS definition [1], which was used in the present study. In this definition, presence of any three of five given risk factors constitutes a diagnosis of MetS. For abdominal obesity, the IDF cut-off points were used (waist circumference ≥94 cm in men and ≥80 cm in women) [60]. For the other components, the cut-off points were as follows: S-triglycerides ≥150 mg/dL (1.7 mmol/L), S-HDL cholesterol <40 mg/dL (1.0 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women, S-glucose ≥100 mg/dL (5.5 mmol/L), systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mm Hg. The glucose measured in Tromsø 4 and 6 was non-fasting.

3.3.2 Metabolic score

Most studies investigating MetS have been dichotomized, and using a continuous metabolic score placing equal weight on each criteria has been recommended [61, 62]. Participants were given a score from zero to five for each fulfilled criterion of MetS based on the JIS definition [1], and the total score is referred to here as the metabolic score. The cut-off points were the same as those used to define metabolic syndrome.

3.4 Statistical analyses

3.4.1 Statistical analyses Paper II

Based on hormonal difference between men and women and changes in the body over a lifespan (pre- and postmenopausal women, aging), data was presented stratified by gender and age group. Age groups were defined as <45 years, 45–59 years and 60–70 years [63]. Fish consumption was analyzed as a categorical variable both as less/more than once a week and as never, <1, 1, 2–3 or more per week. MetS components were analyzed both as continuous variables and as categorical variables using the JIS definition [1]. To investigate crude associations between fish consumption and MetS prevalence or its components, chi-square tests for categorical variables and analysis of variance (ANOVA) for continuous variables were used. Correlations between pairs of continuous variables were assessed with Pearson's correlation.

Linear regression models were fitted to examine relationships between components of MetS (continuous) as a dependent variable and fish consumption (categorical) as an independent variable. Logistic regression models were used for categorical dependent variables, and potential confounding variables (gender, education, physical activity, single/living with a spouse, parity and lactation) were adjusted for. Parity and lactation were only adjusted for among women. A P-value <0.05 was considered statistically significant. All tests were two-sided. Analyses were performed using IBM SPSS Statistics 22.

3.4.2 Statistical analyses Paper III

Based on hormonal differences among men and women, together with changes over lifespan (pre- and postmenopausal women, aging), data were presented stratified by gender and age groups (<45 years, 45–59 years, 60–69, ≥70 years) [63]. Fish consumption was analyzed both as less/more than once a week, and as 0–1 times/month, 2–3 times/month, 1–3 times/week, 4–6 times/week and 1–2 times/day. MetS components were analyzed both as continuous variables, and as categorical variables using the JIS definition [1].

To investigate crude associations between fish consumption and MetS prevalence or its components, chi-square tests for categorical variables and ANOVA for continuous variables were used. Correlations between pairs of continuous variables were assessed with Pearson's correlation coefficient.

Linear regression models were fitted to examine the relationship between components of MetS (continuous) as a dependent variable and fish consumption (categorical) as an independent variable. Logistic regression models were used when analyzing MetS as a binary outcome variable (MetS no/yes) and potential confounding variables (age, physical activity, cod liver oil, parity and lactation) were adjusted for. Parity and lactation were only adjusted for among women. A P-value <0.05 was considered statistically significant. All tests were two-sided. Analyses were performed using IBM SPSS Statistics 22.

3.4.3 Statistical analyses Paper IV

To investigate crude associations between fish consumption and the MetS components, chi-square tests for categorical variables and ANOVA for continuous variables were used, both with fish consumption as a binary variable (less than once a week/once a week or more) and as a categorical variable (never, <1, 1, 2–3 or more). Correlations between pairs of continuous variables were analyzed using Pearson's correlation coefficients.

Linear mixed models were used to examine changes in the components of MetS and metabolic score, over the 13-year follow-up period, with fish consumption as a categorical independent variable (less than once a week/once a week or more). When examining changes in metabolic score, linear mixed models were fitted with metabolic score (continuous variable) as the dependent variable, with fish consumption (fatty, lean and processed) and time (from 1994–1995 to 2007–2008) as factors (categorical variables), and age as a covariate (continuous variable) (Model 1).

When examining changes in the MetS components, linear mixed models were fitted separately with each of the MetS components modeled as a continuous dependent variable, with fish consumption (fatty, lean and processed), and time (from 1994–1995 to 2007–2008) as factors, and age as a covariate (Model 1). Further, multiple linear mixed models were fitted

with fish consumption (fatty, lean and processed), cod liver oil/fish oil capsules, vitamin D, smoking and time (from 1994–1995 to 2007–2008) as factors, and age, estimated intake of energy and alcohol, education and leisure time physical activity as covariates (Model 2).

One of the major strengths of our study was the possibility to follow individuals from Tromsø 4 to Tromsø 6. To adjust for dependencies caused by the same individuals being measured several times we fitted mixed models for repeated measures. Moreover, using this statistical methodology we did not need to perform any imputation of missing values and used all available information, not just individuals with complete data – which could possibly lead to bias. In total, data on at least one measurement were available for 4,528 participants for waist circumference, 9,029 participants for triglycerides, 9,020 participants for HDL cholesterol, 9,033 participants for BP (systolic blood pressure and diastolic blood pressure), and 4,662 participants for blood glucose.

A P-value <0.05 was considered statistically significant. All tests were two-sided. All analyses were considered exploratory so no correction for multiple testing was performed. All analyses were performed using IBM SPSS Statistics 23.

4 Main results

Paper I

Fish consumption and its possible preventive role on the development and prevalence of metabolic syndrome - a systematic review

In this review, seven studies from Croatia, Finland, France, Iceland, Iran, Korea and the United States were included after identifying 502 literature citations in a PubMed search and assessing 49 full-text articles for eligibility. Four of the included studies (one follow-up and three cross-sectional) found associations between fish consumption and MetS (three among men and one among women). These results suggest that fish consumption may prevent or improve metabolic health and have a protective role in MetS prevention, possibly related to gender, where men may benefit more from fish consumption.

However, due to the small number of included studies, little information regarding the associations between fish consumption and MetS was found.

Paper II

Associations between fish consumption and metabolic syndrome. A large cross-sectional study from the Norwegian Tromsø Study: Tromsø 4

The prevalence of MetS was 8.1% in the whole sample. In this sample of individuals aged 26–70 years from Tromsø 4 (1994–1995), fish consumption once a week or more was associated with a lower risk of having MetS compared to fish consumption less than once a week (OR 0.64, 95% CI 0.45–0.91) among those aged 60–70 years. When investigating fatty and lean fish separately, lean fish consumption once a week or more was associated with a reduced risk of having MetS among those aged 60–70 years when compared to those consuming lean fish less than once a week (OR 0.65, 95% CI 0.48–0.87). No association was found for fatty fish consumption or for lean fish consumption in the younger age groups (<45 and 45–59 years). Higher fish consumption (≥1/week) was associated with a healthier lipid profile, with increased HDL cholesterol and decreased triglycerides in adjusted models.

These findings support the hypothesis that fish consumption may be associated with a lower risk of MetS and that fatty and lean fish consumption may influence MetS risk differently, possibly related to age.

Paper III

Lean fish consumption is associated with lower risk of metabolic syndrome: a Norwegian cross sectional study

Based on data from the Tromsø 6 survey (2007–2008), the prevalence of MetS was 22.6% in the whole sample. When investigating the consumption of fatty and lean fish together, fish consumption once a week or more was associated with a lower risk of having MetS compared to fish consumption less than once a week, for men in the crude model (OR 0.85, 95% CI 0.74 to 0.98). In the age-adjusted model, a lower risk of having MetS was found among those consuming fish once a week or more compared to those consuming fish less than once a week, for both women (OR 0.83, 95% CI 0.71 to 0.97) and men (OR 0.81, 95% CI 0.73 to 0.90).

In age-adjusted models, lean fish consumption once a week or more was associated with a decreased risk of having MetS compared to lean fish consumption less than once a week, both in the whole sample (OR 0.87, CI 95% 0.79 to 0.96) and among men (OR 0.85, CI 95% 0.74 to 0.97). This association was only found among women after further adjusting for physical activity, cod liver oil, parity and lactation (OR 0.85 CI 95% 0.72 to 0.99). Fatty fish consumption was not associated with a decreased risk of having MetS in any of the models.

Increased consumption of both fatty and lean fish consumption (0–1 times per month, 2–3 times per month, 1–3 times per week, 4–6 times per week, 1–2 times per day) were associated with a healthier lipid profile, decreased serum triglycerides, and increased HDL cholesterol in the whole sample. However, for lean fish the associations were only significant among women, in the age-adjusted model for triglyceride and in the crude model for HDL cholesterol.

Our findings suggest that lean fish consumption may contribute to a lower risk of having MetS and that lean fish consumption has a place in MetS prevention.

Paper IV

Lean Fish Consumption Is Associated with Beneficial Changes in the Metabolic Syndrome Components: A 13-Year Follow-Up Study from the Norwegian Tromsø Study.

In this follow-up study, dietary data from the Tromsø 4 survey (1994–1995) and MetS data from both the Tromsø 4 survey (1994–1995) and the Tromsø 6 survey (2007–2008) were used.

During the 13-year follow-up period, several of the MetS components changed significantly, and waist circumference, systolic blood pressure, and blood glucose increased among both genders. Triglycerides increased significantly for women and decreased significantly for men; HDL cholesterol increased only for men. A decrease in diastolic blood pressure during the follow-up period was also identified; however, this change was only significant in women. Additionally, mean metabolic score increased significantly for both genders.

During the 13-year follow-up period, lean fish consumption once a week or more was significantly associated with decreased future metabolic score, decreased triglycerides, and increased HDL cholesterol, whereas decreased waist circumference and blood pressure was identified only for men (age adjusted models). The associations remained statistically significant after further adjustments, except for triglycerides among women. Fatty fish consumption was significantly associated with increased waist circumference for both genders and increased HDL cholesterol levels in men. However, only the association with waist circumference remained statistically significant after further adjustments.

The results suggest that fatty and lean fish consumption may influence MetS differently and that lean fish consumption in particular seems to be associated with beneficial changes in the MetS components.

5 General discussion

Our overall findings in this study suggest that lean fish consumption is associated with a decreased risk of MetS, together with beneficial changes in the MetS components. In addition, lean fish consumption was associated with lower metabolic score during the follow-up period. Fatty fish consumption, on the other hand, was not associated with decreased risk of MetS or these beneficial changes during the follow-up period. Still, both increased fatty and lean fish consumption is associated with a healthier lipid profile, with decreased serum triglycerides and increased HDL cholesterol.

5.1 Methodological considerations

Epidemiological studies aim to be valid and reliable and attempt to avoid both random and systematic errors [64]. When interpreting these findings, it is important to consider how much the observed associations may be affected by errors in design, conduct or analysis [65]. Interpreting the findings therefore requires consideration of bias, confounding and chance [65]. The validity of a study refers to both internal and external validity, where internal validity refers to inferences drawn from the sample population, and external validity refers to the population outside of the sample population and concerns generalizability [64]. Internal validity is considered a prerequisite for external validity, and violations such as selection bias, information bias (measurement problems) and confounding can be a threat to internal validity [64].

5.1.1 Selection bias

Selection bias arises when the association between exposure and outcome differs between attendees and non-attendees [64]. In the Tromsø study, whole birth cohorts from the municipality of Tromsø were invited to participate, via personal invitations based on the official population registry [56]. Attendance rates were high, with 72% in Tromsø 4 and 66% in Tromsø 6. The slightly lower rate in Tromsø 6 was due to a lower attendance rate among the relatively young and those who had never participated previously [56]. Still, the surveys

had a high attendance rate compared to similar studies in Norway conducted during the same period [66, 67]. The age distribution of the attendees in Tromsø 4 and 6 is described below (**Figure 7 and 8**).

Selection bias is less of a problem in cohort studies, such as the Tromsø study, because the enrollment of exposed and non-exposed is done prior to the possible development of the outcome of interest. Another issue is the sample size, which ensures that a study has enough power (large enough sample size) to detect effects of interest [65]. In our study, sample size determination was not necessary due to the large sample size. Furthermore, the sample size was large enough in all age groups to perform the statistical analyses. Still, larger sample sizes increase the power during analysis and may reveal statistical differences that are not clinically relevant. We therefore were more concerned with type II error (failing to reject the null hypothesis when it is false), given the very large sample size. Furthermore, given the large sample size, many of our findings could be statistically significant (P-values <0.05) but not clinically relevant or meaningful. Therefore, we present our estimates with 95% CI, which makes it easier to interpret them. The role of chance has been assessed by performing statistical significance tests and calculating confidence intervals. The statistical significance test yields the probability (P-value) that the result is due to by chance alone. In our study, a P-value <0.05 was considered statistically significant. This means that if the P-value is greater than 0.05, it is thought that chance cannot be excluded as an explanation for the observed association. However, when interpreting P-values in such a large sample, small effects that may be irrelevant may achieve statistical significance. Therefore, confidence intervals are more informative because they provide a range of values for the exposure–outcome association, which in our study is the fish consumption–MetS/metabolic score.

In cohort studies, loss of follow-ups may also be a cause of concern. However, of the participants in Tromsø 6, 80% also attended in Tromsø 4 [57].

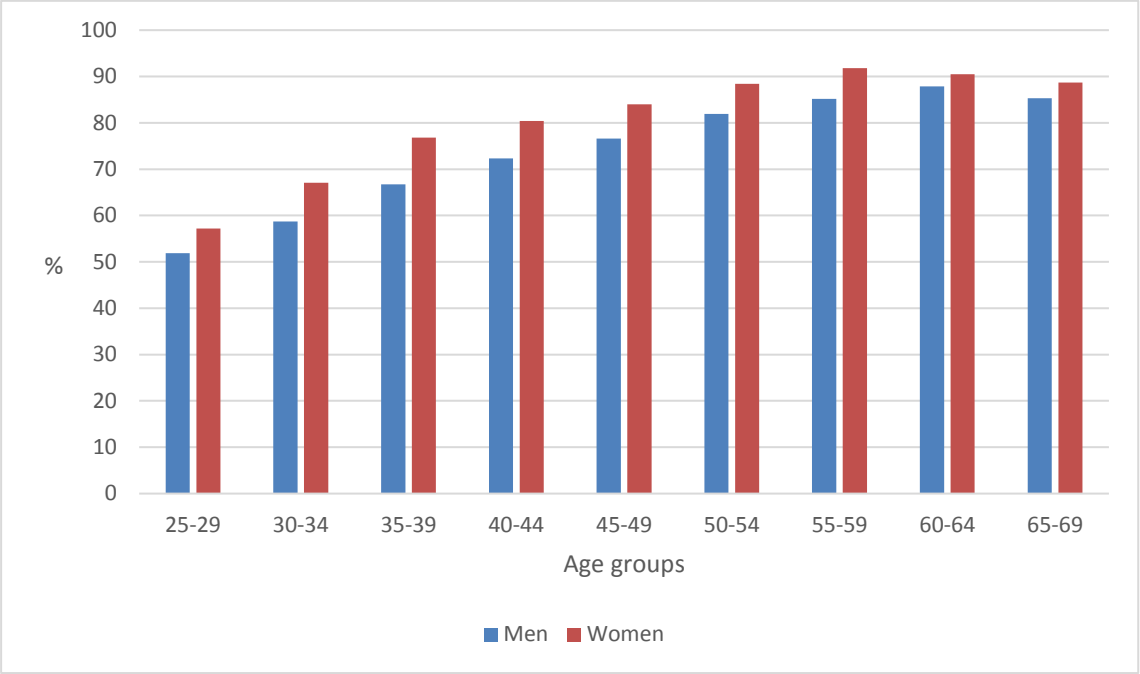

Figure 7. Attendance by age groups among men and women in Tromsø 4 [68].

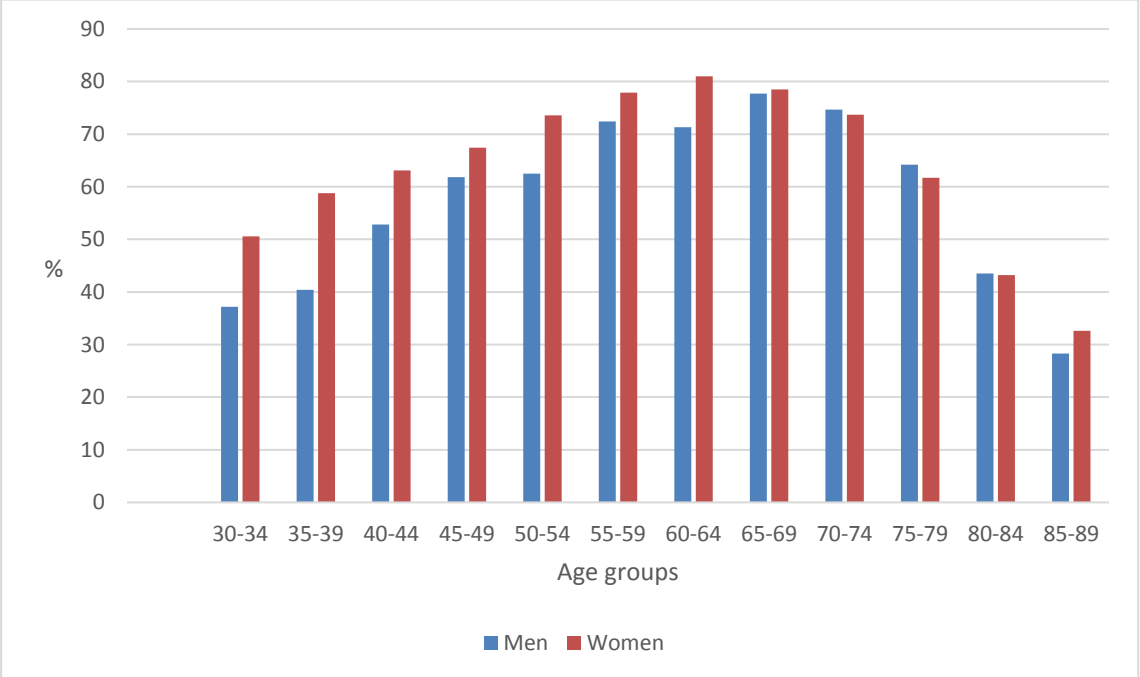

Figure 8. Attendance by age groups among men and women in Tromsø 6 [68].

5.1.2 Information bias

Information bias such as recall bias, measurement errors and misclassifications of information may occur when information is gathered from participants [64]. This may lead to changed direction and magnitude in the associations revealed. For discrete variables such as gender or fish consumption in Tromsø 4 and 6 (Papers II–IV), data were collected through questionnaires, measurements and blood samples. MetS and metabolic score were defined using the JIS definition [1].

Questionnaires

Self-administered questionnaires are often used in population-based studies such as the Tromsø study because of their many advantages. They are cost-effective, timesaving and well-suited to large studies assessing dietary intake among thousands of subjects. Self-administered questionnaires have also been found to be more accurate when collecting sensitive information [69]. Furthermore, self-administered questionnaires have been found to be less biased compared to interviewer-administered questionnaires, as respondents have been found to give more socially acceptable answers when they are interviewed [70]. However, both recall bias and misclassifications may occur. Recall bias can be both intentional and un-intentional. It is possible that some of the participants did not remember correctly or were underreporting. However, participants may be more likely to underestimate unhealthy food consumption and overestimate healthy food consumption. Still, high reproducibility has been observed when using FFQs to assess key nutrients of a Mediterranean diet in a Quebec population [71]. Nevertheless, we have no reason to believe that fatty fish consumption is more/less often reported than lean fish consumption or that women underreport more/less than men do.

Misclassifications can be classified as differential (dependent on other study variables) or non-differential (independent of other study variables); they are classified as differential if the exposure differs between those with and without the outcome, while they are classified as non-differential if the misclassification is independent of the outcome [64]. Fish consumption was assessed using self-administered, semi-quantitative questionnaires (Papers II–IV), which is considered a valid method when assessing fish consumption [72-75]. However, fish consumption may be under- or over-reported and is additionally a modifiable exposure, which

can lead to non-differential misclassifications. In addition, examples of lean fish are not given in the Tromsø 6 questionnaire, whereas for fatty fish examples are provided of different species, which may lead to differential misclassification and under- or over-reporting of fish consumption. Furthermore, when reporting information retrospectively, there is an increased risk of recall bias.

We analyzed fish consumption as a continuous variable when modelling lean and fatty fish separately. However, when we merged lean and fatty fish consumption, it would not have been meaningful to treat the resulting variable as continuous. Therefore, fish consumption (both lean and fatty) was categorized as low consumption (less than once a week) and as high consumption (once a week or more often). This is clearly a limitation of our study, but given our data, we had to make a simplification.

Another weakness in our study is that there is an overlap between those consuming fatty fish and those consuming lean fish. This was because most participants consumed both lean and fatty fish; however, the consumption was most likely in different quantities. Therefore, additional effects from consuming both fatty and lean fish cannot be ruled out. This study also has data only on the frequency of fish consumption and not on portion size, which may have also influenced the results. Furthermore, the high level of fish consumption among participants in the present study may have limited the possibility of investigating the effects of smaller amounts of fish consumption.

Measurements and blood samples

The measurements used in our study were measurements of waist circumference and blood pressure in addition to blood samples (triglyceride, HDL cholesterol and glucose). All examinations, measurements and laboratory work followed standardized procedures and were carried out by trained health personnel [56, 57], which increases data reliability. In addition, several different anthropometric measures to assess body fat distribution were taken of participants in the Tromsø study, including waist circumference, weight and body mass index (BMI). This makes it possible to investigate the validity of waist circumference as a measure of obesity through correlation analysis. We found that the validity was generally high, with a high correlation between waist circumference, weight and BMI (**Table 5**).

In the Tromsø study, waist circumference was measured at the belly button without outerwear, using a measuring tape [57]. Measuring waist circumference can be challenging, especially among obese participants [25]. Although waist circumference is correctly measured at the level of the belly button, in obese individuals the belly button can be directed downward because of excessive abdominal fat (**Figure 1**). Therefore, a more correct measure can be obtained by measuring circumference at the midpoint between the lower border of the rib cage and the iliac crest [25]. This may lead to measurement problems, at least in obese individuals. Furthermore, the use of waist circumference to assess abdominal adiposity has been questioned, and it has been suggested that sagittal abdominal diameter SAD might be a better measure of adiposity, at least regarding the prevalence of dysglycemia [76] (**Figure 1**).

Table 5. Pearson's bivariate correlation analysis of waist circumference.

	Waist circumference	
	Tromsø 4	Tromsø 6
Weight (kg)	0.86**	0.84**
BMI (kg/m2)	0.77**	0.81**

** Correlation is significant at the 0.01 level (2-tailed).

In our study, we used the IDF criteria for waist circumference when diagnosing MetS. This may have led to a higher prevalence of MetS than if we had used higher cut-off points. When higher cut points are used to diagnose MetS, fewer individuals will be identified as having the syndrome than when using the lower cut-off points [1]. This use of different inclusion criteria also makes it difficult to compare studies.

For blood pressure, the first of three measurements was used in our analysis. However, all three measurements did have high correlations (**Table 6**). Nevertheless, the use of the first blood pressure measurement may have influenced our results. First measurements tend to give higher values due to "the white coat effect", wherein there is a rise in blood pressure in the presence of a medical practitioner. However, this response is observed across all categories of blood pressure from normotensive to hypertensive subjects [77].

Table 6. Pearson's bivariate correlation analysis of blood pressure measures.

	Systolic blood pressure 1		Diastolic blood pressure 1	
	Tromsø 4	Tromsø 6	Tromsø 4	Tromsø 6
Systolic/diastolic blood pressure 2	0.92**	0.94**	0.89**	0.90**
Systolic/diastolic blood pressure 3	0.90**	0.93**	0.88**	0.89**

** Correlation is significant at the 0.01 level (2-tailed).

In this study, we did not have data for fasting blood glucose, as is often the case in large studies, due to the inconvenience of fasting to participants. It would have been a great advantage to have fasting blood glucose measurements available; however, it was not possible to obtain more information beyond the variables already collected in our data file. Therefore, we analyzed the measurements as they were collected, and pointed out that this is a limitation of the study. In addition, C-peptide levels, that releases when insulin is produced, would be of interest because high levels are found among insulin resistant and DM2 subjects. However, data on C-peptide levels among the participants were not available in the data set and therefore could not be included and analyzed.

5.1.3 Confounding

Associations between exposure and outcome can be obscured by confounding variables [78]. This may lead to an under- or overestimation of an effect and may also change the direction of the observed effect. Investigators therefore try to prevent or control for confounders. A simple definition of confounding can be a mixing of effects, implying that the effect of the exposure is mixed together with the effect of another variable [64]. Therefore, it requires knowledge to identify confounders of possible associations between exposure and outcome. For a variable to be a potential confounder, it must be associated with the exposure and also be an independent risk factor for the outcome [65]. However, it must not be on the causal pathway between the exposure and the outcome (e.g., fish consumption–MetS). In such cases it would be a mediator/moderator. Nevertheless, any factor that is believed to have a real effect on the outcome is a potential confounder, and both factors that have a direct causal link and proxy measures of more direct unknown causes such as age can be confounders [65].

Confounding can be dealt with when a study is designed and when the results are analyzed if data on possible confounders has been collected [65]. However, excluding all bias due to confounding is not possible. It is, though, possible to control for confounders to a certain extent, and different techniques may be applied to prevent or control for confounding. When designing an epidemiological study randomization, restriction and matching can be used [65]. However, the latter can only be used in case-control studies and it has been argued that matching can never eliminate all confounding. Therefore, modern model based methods are a preferred choice for dealing with confounding, especially when a large dataset is available.

In our study, we used data from the Tromsø study, in which whole birth cohorts and random samples from the Tromsø population were invited to participate [56, 57]. Our study was a population-based survey, so randomization by fish consumption would not be feasible with such a large sample size. However, despite not being randomized by fish consumption, we assume that the distribution of possible confounding variables was similar in both groups. In addition, all our analyses were adjusted for known and available possible confounders.

Restriction is a procedure often used to limit participation to subjects who are similar in relation to the confounder [65]. In our study, we restricted the sample to those below the age of 70 years from Tromsø 4 (1994/95) (Papers II and IV) for several reasons: to create a more homogeneous dataset and to decrease the loss of follow-up due to high age (Paper IV). Moreover, in Tromsø 4 participants below the age of 70 years received a separate questionnaire, that included questions on fatty, lean and processed fish consumption (Papers II and IV).

Stratification is a technique in which the strength of the association is measured separately within a defined stratum of the confounding variable [65], such as age. In our study, we stratified by gender and age groups (Papers II, III and IV) [63] due to hormonal differences between men and women and to accommodate for changes that occur in the body over a lifespan (pre- and postmenopausal women, aging). The investigated associations were then estimated separately for each age group/gender.

To further control for confounding, we used statistical modelling such as multiple regression modelling. The selection of possible confounding variables was based on a priori knowledge. Parity and lactation [27] were adjusted for among women, and use of cod liver oil/ fish oil capsules [79] and vitamin D [80] were adjusted for because it might improve features of

MetS. In paper IV, we also mutually adjusted for consumption of lean fish, fatty fish and processed fish, in addition to other potential confounders that were included in the regression models (smoking [81], physical activity, energy intake, alcohol intake, education). However, despite our best efforts, there may be potential risk factors that are not adjusted for, such as different food variables to which we did not have access.

5.1.4 External validity

Internal validity is considered a prerequisite for external validity [64], and despite the limitations discussed above, the overall consideration of internal validity of our study should be satisfactory. A major concern for consideration of external validity is representativeness. This study (Papers II–IV) uses data from the Tromsø study, which was conducted among a representative sample from the Tromsø population. With an overall high attendance rate (Tromsø 4: 72%, Tromsø 6: 66%), we believe the samples from the Tromsø study are representative of the Norwegian population, as it is largely a Caucasian population with limited ethnic diversity [56, 57].

However, as the non-attendees tended to be younger, less educated men compared to the attendees [56, 57], we may be missing information from young, unmarried, less educated men who also may have a more unhealthy diet [58]. In addition, one might argue that fish consumption is higher among individuals living in the Tromsø area compared to those living in the rest of Norway. Fish consumption has been observed to be as much as 30% higher in Northern Norway compared with the average consumption of fish in other parts of Norway (Norkost 1997) [46]. On the other hand, a similar proportion of energy from different sources (fats, protein, carbohydrates, alcohol) has been reported in Tromsø 4 and the Norkost survey [58], a nationwide food consumption survey among adults in Norway. In our study (Paper II), we found that those with the highest fish consumption were older than those with lower fish consumption (Tromsø 4). This was also reported in Norkost 1997 for the same time period [46]. Based on the above considerations, we therefore regard our results to be representative for the general Norwegian population, thus assuring a high level of external validity of our data.

5.2 Discussion of main findings

After reviewing the current literature (Paper I), associations between fish consumption and MetS and its components were investigated among adults at two different time points (1994–95 and 2007–2008) (Papers II and III). Furthermore, possible associations between fish consumption, both fatty and lean, and changes in MetS components during a 13-year follow up period were investigated, including changes in metabolic score (Paper IV).

The main finding from this large population-based study is that lean fish consumption is associated with a decreased risk of MetS, and beneficial changes in four out of five components comprising MetS (waist circumference, triglycerides, HDL cholesterol, blood pressure). However, some of the identified associations were only statistically significant for men (waist circumference and blood pressure). Nevertheless, lean fish consumption was also associated with a decrease in future metabolic score for both genders.

These findings support the hypothesis that higher fish consumption is associated with a lower risk of MetS and a healthier metabolic profile. However, fatty and lean fish consumption may influence MetS risk differently, possibly also related to age and gender.

This study adds valuable information to the body of knowledge about fish consumption and MetS. However, due to its design, the results from this study may be used to generate further hypotheses that can be tested in other study designs.

5.2.1 Fish consumption and metabolic syndrome

In the sample population from Tromsø 4 (1994–1995), fish consumption (fatty and lean) once a week or more was associated with a lower risk of having MetS among those aged 60–70 years when compared to fish consumption less than once a week (OR 0.64, CI 95% 0.45 to 0.91) (Paper II). However, when investigating fatty and lean fish separately, this significant association was only found for lean fish consumption and not for fatty fish consumption (Paper II).

When these associations were investigated in the sample population from Tromsø 6 (2007–2008), men consuming fish once a week or more had a 15% lower risk of having MetS

compared to those consuming fish less than once a week (OR 0.85, CI 95% 0.74 to 0.98). However, in the age-adjusted model, this association was revealed for both women (OR 0.83, CI 95% 0.71 to 0.97) and men (OR 0.81, CI 95% 0.73 to 0.90) (Paper III).

When reviewing current literature investigating fish consumption and the development or prevalence of MetS (Paper I), only scarce information was found due to few studies were included in our review. In addition, three of the four studies that reported associations between fish consumption and MetS were cross-sectional, and thus only captured a single point in time. Therefore, any possible causal effect could not be determined. Nevertheless, cross-sectional studies may be used to provide information regarding possible diet–disease relations. However, four of the seven included studies (one follow-up and three cross-sectional) found associations between fish consumption and MetS, suggesting that fish consumption might have a protective role in MetS prevention. This is in agreement with a recent meta-analysis that found a significant inverse association between fish consumption and the incidence of MetS when pooling data from prospective cohort studies [82]. However, they found no significant association between fish consumption and MetS after pooling the cross-sectional studies [82].

Of the seven studies included in our review (Paper I), associations between fish consumption and MetS were found in the follow-up study from Korea [15] and in three cross-sectional studies [16, 17, 83], whereas only one of these studies found associations among women [83], suggesting that this possibly protective role might be related to gender [84].

In the follow-up study from Korea, associations between average frequency of fish consumption (sum of dark- or white-meat fish and canned tuna) and the incidence of MetS were investigated [15]. Both male and female Koreans (n=3504) aged 40–69 years were included. MetS was defined according to the definition from the Adult Treatment Panel III (ATP III) [60], except for waist circumference, for which alternative criteria were used for the appropriate waist cut-off points [85]. After excluding participants with MetS at baseline, they found that the adjusted risk of having MetS decreased to less than half (OR 0.43, 95% CI 0.23 to 0.83) among men who consumed fish daily compared with those who consumed fish less than once a week [15]. Also, a recent 25-year follow-up study found that fish consumption (non-fried) was inversely associated with the incidence of MetS later in life among 4,356 American young adults who were free from MetS and diabetes at baseline [86].

The Finnish cross-sectional study (n=1,334), found that consumption of fish was inversely associated with MetS among men when investigating associations between different foods and nutrients and the risk of having MetS [16].

In the French population, consisting only of men aged 45–64 years, the relation between food groups and the frequency of insulin resistance syndrome (ATP III) was examined [17]. A negative association between insulin resistance syndrome and fish consumption was found, and the proportions of insulin resistance syndrome decreased along tertiles for fish consumption (adjusted OR 0.57, 95% CI 0.38 to 0.86) in the highest tertile of fish consumption [17].

The cross-sectional study from Iran [83] aimed to identify associations between fish consumption and MetS and its components among women; participants were female nurses (aged >30 years) randomly selected from various hospitals (n=420). MetS was defined according to the JIS definition [1]. High fish consumption was inversely associated with MetS, where women in the highest tertile of fish consumption were less likely to have MetS compared with their counterparts in the lowest tertile (OR 0.35, 95% CI 0.14 to 0.88) [83].

5.2.2 Fish consumption and components of metabolic syndrome

In our cross-sectional studies (Papers II and III), both increased fatty and lean fish consumption were associated with decreased serum triglycerides and increased HDL cholesterol. For the other components of MetS, our results were inconclusive.

Decreased triglycerides and increased HDL cholesterol were also observed among men in the Korean follow-up study; however, no significant association was found among women in that study [15]. In a population in Cyprus and the islands of Mitilini and Samothraki in Greece, an inverse association between fish consumption and triglycerides has been observed [11]. Long-term fish consumption has also been associated with a better lipid profile in an elderly population from Mediterranean islands (aged 65 to 100 years) [11]. However, the amounts and types of fat in the diet have been shown to influence plasma lipid levels [87]. A healthier lipid profile has also been observed in obese populations when an intervention group was given a healthy diet containing fish [88, 89]. It has been suggested that an association between

consumption of fish and the incidence of MetS may be driven by triglyceride and HDL cholesterol levels [15]. In an intervention study, short-term intake of both lean and fatty fish decreased triglyceride levels in healthy subjects, and HDL cholesterol levels increased after intervention with fatty fish (salmon) [90].

It has been reported that adults with low HDL cholesterol levels may be more susceptible to developing MetS over time [91]. Associations between fish consumption and increasing HDL cholesterol levels have also been observed in other studies; however, most studies investigated total fish consumption, assessing fatty and lean fish as an entity and not separately. Increased HDL cholesterol levels have been observed in an intervention study after participants received fatty fish (salmon) for two weeks [90]. The amounts and types of fat in the diet, such as fat from consumption of fatty fish, may influence plasma lipid levels [87]. We also observed that higher lean fish consumption was associated with higher HDL cholesterol levels (Paper III). A recent study found reduced urinary excretion of metabolites involved in mitochondrial lipid and energy metabolism among healthy subjects consuming a diet in which 60% of the dietary protein was from lean-seafood (lunch and dinner meals including cod, pollack, saithe and scallops), suggesting that higher lipid catabolism may occur after lean seafood consumption [92].

Other studies have observed improvement in the other MetS components. A decrease in waist circumference was observed among men receiving fish or fish oil (cod, salmon or fish oil) ($P<0.05$) compared to those who did not receive fish or fish oil in an eight-week intervention study (n=324, 20–40 years of age, BMI 27.5–32.5kg/m) conducted in Iceland, Spain and Ireland (SEAFOODplus YOUNG study) [93]. A lower waist circumference has also been observed among Europeans (n= 497,308; 71% women) with a higher adherence to a Mediterranean diet where fish and seafood were included [94]. However, the European Prospective Investigation into Cancer and Nutrition (EPIC) study found no preventing effect of lean fish consumption on waist circumference [95].

Long-term fish consumption has been associated with lower blood pressure in the Mediterranean islands; however, this was in an elderly population (aged 65 to 100 years) [11]. Fish consumption has also been found to improve blood pressure in intervention studies both among young adults in Europe [96] and among cardio heart patients in Finland where lean fish consumption at least four times per week reduced blood pressure levels [97].

An inverse association between fish consumption and fasting blood glucose has been observed in populations in Cyprus and islands in Greece [11]. Improved glucose metabolism has also been seen in obese populations when an intervention group was given a healthy diet containing fish [88, 89]. In our study (Paper II), slightly higher blood glucose levels were observed among those consuming fish. However, blood glucose in our study was non-fasting, which may have influenced the results.

5.2.3 Nutrients in fatty and lean fish

Several mechanisms may partly explain the beneficial effects of fish consumption on the components of MetS, which may work through reduced ectopic fat accumulation, improved lipid metabolism, and hypotensive effects. Fish contains a variety of nutrients that may contribute to positive health implications with regard to MetS [49, 50]. Fatty fish contains more fat and different fatty acids compared to lean fish, while lean fish contains more iodine and less energy compared to fatty fish [48]. However, the evidence has been sparser considering the health effects of lean fish such as cod, which may contribute to better cardio-metabolic health.

Proteins in fish

Lean fish such as cod is considered a superior source of proteins; however, little is known about possible differences between the proteins in lean fish and fatty fish. Dietary proteins regulate lipid metabolism and have been found to slow both the absorption and the synthesis of lipids and to further promote lipid excretion [98]. Beneficial effects of fish proteins on both hyperlipidemia [99] and insulin sensitivity [100] have been revealed among those consuming fish proteins from cod, compared to consuming other animal proteins. Animal studies have previously suggested that protein from fish may influence plasma and liver lipids [101], and cod protein prevented rats from developing skeletal muscle insulin-resistance, compared to rats fed soy protein and casein [102]. Dietary proteins may improve lipid profiles through mechanisms that may differ based on the dietary protein source, together with their quantity

and composition [98]. Indeed, some studies have observed hypocholesterolemic activity of fish proteins that may affect the absorption and excretion of lipids [98].

A comparison of energy and nutrient intakes between fish and red meat dinners showed that fish dinners contained less energy and had a higher percentage of energy from protein than meat dinners [103]. Furthermore, plasma bile acid levels may be modulated by the dietary protein source. In high fat-treated rats, increased levels of plasma bile acids were associated with a significant reduction in diet-induced obesity together with increased whole body energy expenditure and dissipation of energy in the form of heat [104].

Furthermore, both the digestion and absorption of proteins may influence how proteins affect metabolic processes [105]. Different approaches to defining protein requirements and quality have been considered [106], as has an approach to defining slow- and fast-digested dietary proteins that may modulate postprandial protein and glucose metabolism differently [105]. Furthermore, a greater satiety level has been observed among participants consuming fish compared to participants consuming beef or chicken consumption [107].

Fish consumption may also be associated with inflammatory processes. Decreased CRP has been observed among participants receiving dietary advice plus either 300 g fatty fish (salmon) or 300 g lean fish (cod) per week for six months, when compared with the dietary advice only group (salmon: -0.5 mg/L, 95% CI -0.9 to -0.2, cod: -0.4 mg/L, 95% CI -0.7 to 0.0) [14]. However, those in the fatty fish group were healthier at baseline compared to those in the lean fish and dietary advice group [14]. In a rat model, fish proteins have been observed to decrease the production of pro-inflammatory cytokines such as TNF-α and IL-6 [108]. Increased levels of IL-6 and CRP have previously been observed among hypertensive participants [109].

Taurine

Fish protein contains amino acids such as taurine; however, the concentration of taurine in fish muscle may vary among different fish species. The highest concentrations have been found in lean fish [52]. Higher plasma taurine concentrations have been observed among participants after meals consisting of 50 g protein from fish compared to proteins from beef or chicken [107]. The effect of taurine on the components of MetS has previously been

reviewed, and beneficial effects on obesity and lipid profiles and a hypotensive effect have been observed [110]. Furthermore, beneficial effects of taurine on blood lipids have also been found in an intervention study among participants receiving a combination of taurine and n-3 when compared to those receiving n-3 alone [111]. Taurine has also been observed to decrease CVD risk through a variety of mechanisms such as improving lipid profiles and antagonism of Angiotensin II action influencing the blood pressure [112]. Taurine supplementation has also been observed to decrease triglyceride among humans [113], possibly due to a decrease in very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol [114]. Furthermore, taurine has been identified as having an anti-inflammatory effect, possibly through suppressing the secretion of TNF-α and other proteins in the inflammation pathways [110].

Taurine is an exclusively free amino acid, and the content of taurine may be influenced by different industrial processes. When compared with the taurine content of freshly caught fish specimen, the loss of taurine in processed products may range up to 100% [115].

N-3 fatty acids

The marine n-3 fatty acids EPA and DHA are present mainly in fatty fish [116] but are also in lean fish. However, cod liver and cod liver oil contain large amounts of n-3 fatty acids and vitamins such as vitamin D [117]. EPA and DHA are responsible for biological actions such as maintaining the cell membrane, inhibiting inflammatory processes and decreasing the secretion of pro-inflammatory cytokines [118]. A higher proportion of lean fish consumers reported using cod liver oil/fish oil capsules, compared to the fatty fish consumers (Tromsø 4). However, in the multiple models, this was adjusted for (cod liver oil/fish oil capsule use) (Paper IV). Cod liver oil may have potential anti-inflammatory effects [119] and thus be beneficial in the treatment of conditions with an inflammatory component, such as MetS [120]. Moreover, the use of n-3 fatty acids is considered a valuable clinical tool in the treatment of hypertriglyceridemia [121], and DHA in particular has been favorably associated with decreased triglycerides [122-125] as well as increased HDL cholesterol [123, 126, 127]. Fish oil supplementation has also been associated with lower blood pressure [126]. However, it has been argued that n-3 fatty acids may be more efficacious when consumed in fish than equivalent amounts provided as fish oil capsules [128], and n-3 FAs from fish and sea

mammals have been associated with lower blood pressure and serum triglycerides [129]. Furthermore, a reduced risk of MetS among men with a high n-3 fatty acid intake has been observed in a prospective Korean study when compared with those with low intake (OR 0.53, 95% CI 0.28 to 0.99) [15]. N-3 PUFAs have been shown to regulate pathways controlling fat storage and fat mobilization and to decrease lipid accumulation processes [130]. However, background diet may influence the ability of n-3 PUFAs to protect against the development of obesity, glucose intolerance and adipose tissue inflammation, and high levels of dietary sucrose have been found to counteract the anti-inflammatory effect of fish oil in adipose tissue and to increase obesity development in mice [131].

Iodine

Fish has the highest natural concentration of iodine [132]. Iodine is important for production of the hormones thyroxine (T4) and triiodinethyroxine (T3) and for the normal functioning of the thyroid gland [48]. However, there is great variation in iodine content between different fish species, and lean fish is the source of nearly all iodine intake from fish [48]. The highest levels of iodine are found in lean fish such as cod (199–130 mcg per 100 g), and the lowest content levels are found in fatty fish such as salmon and trout (5–19 mcg per 100 g) [51]. Among adults, the recommended daily intake (RDI) of iodine is 150 mcg per day [53], which is considered the appropriate amount to allow normal T4 production without stressing the thyroid [133]. Previously, higher thyroid volume has been found among those with MetS, in comparison to those without MetS [134]. Furthermore, lower urinary iodine has been found among obese women, in comparison with healthy non-obese women [135].

Contaminants and other undesired substances in fish

Fish consumption may contribute to consumption of contaminants and other undesired substances, such as methylmercury and POPs, which include dioxins and dioxin-like PCBs [48]. The highest mercury fillet concentrations of the marine fish species are found in the large wild fish species such as halibut, Greenland halibut and tuna. Only roe cod was found to have mercury levels below the limit of quantification (LOQ), whereas all other fish species had levels over the LOQ [48]. Mercury has been associated with an increased risk of

hypertension [136]; however, the possible harmful effects of mercury may be attenuated by high levels of selenium [137]. As for the lipid-soluble contaminants such as dioxins and PCBs, fatty fish is considered to be the main source, contributing to 76% of the exposure from fish [48].

Exposure to POPs may lead to increased levels of serum lipids and to dyslipidemia [138], which is involved in the pathogenesis of DM2 and MetS. Furthermore, PCBs have been associated with a higher risk of hypertension among Inuit from Quebec, Canada [139], and a recent review suggested that the concentration of certain POPs, especially dioxin-related compounds, is associated with the risk of hypertension [140]. Continuous intake of farmed fatty fish has been found to contribute to insulin resistance and obesity in mice, possibly due to the presence of POPs in fatty fish [141]. However, the composition of the dietary macronutrients has been found to modulate the accumulation of POP in adipose tissues in mice, where the intake of POPs in a diet with a high protein:sucrose ratio caused lower tissue accumulation than the intake of POPs with a low protein:sucrose ratio [142]. This might be important when trying to decrease the burden of POPs.

5.2.4 Age and gender differences

We observed gender differences both when investigating fish consumption and MetS (Paper III) and when investigating fish consumption and metabolic score. It is well known that gender differences exist with regard to lipid levels and body-fat distribution, as well as sex hormones. Higher HDL cholesterol has been observed among women compared to men at all ages [143], which is also reflected in the MetS definition [1]. In addition, aging and menopause may lead to differences in lipid profiles [143]; increasing triglyceride levels with increasing age have been reported for both genders [143]. Women have a higher percentage of body fat with more adipose tissue in the hips and thighs compared to men [144]. The major function of adipose tissue is the storage and release of energy. Women, more so than men, may accumulate adipose tissue without metabolic consequences, where gluteal-femoral adipose tissue in particularly may diminish metabolic risk [144]. There are further gender differences in the amount of sex hormones, which also change over a lifespan. Both testosterone and estrogen levels have been associated with components of MetS [143]. Ovarian hormones such as estrogen have been suggested to have a protective role regarding

MetS, at least before menopause [145]. This may be due to estrogen's ability to decrease inflammation and reduce the glucocorticoid response [146]. Premenopausal women have higher estrogen levels, and estrogen's ability to decrease inflammation and reduce glucocorticoid response may inhibit the presence of MetS [146]. In addition, parity, lactation and giving birth to more children seem to influence MetS rates [27, 28, 147, 148]. Estrogen is thought to have an effect on adipose tissue, and it may provide anti-inflammatory effects that may protect women from diseases associated with inflammation of adipose tissue [149]. Men and women also differ in how they regulate energy balance, and estrogen may directly influence energy balance through its regulation of food intake and body adiposity [145]. In our study, when we adjusted for confounders, by a priori knowledge, we also adjusted for parity and lactation among women, because these variables were considered confounders and therefore might affect the results.

5.2.5 Lifestyle, dietary pattern and metabolic syndrome

Lifestyle factors such as diet are useful components in a strategy aiming to improve components of MetS. Previously, beneficial associations between diets containing fish and MetS have been reported, both in follow-up studies with healthy diet [150], and with the Mediterranean diet [151, 152]. This has also been observed in cross-sectional studies [33, 35, 153-156]. Still, not all follow-up [157] or cross-sectional studies [31, 32, 34, 36, 37, 158-162] found an association.

When different healthy foods are consumed together, there may be an additive effect; for example, when fish is consumed together with dairy products and grains, an even more decreased probability of insulin resistance syndrome has been observed compared to consuming fish alone [17]. MetS has previously been associated with an unhealthy lifestyle and with diets high in processed foods such as refined grains, rice, potato chips and pancakes [159]. Lower fish consumption has been observed among participants with an unhealthy lifestyle, such as in a large Danish study (n=48,627) where women with low fish consumption were more likely to be younger (<25 years), nulliparous, smokers and overweight/obese compared to those with high fish consumption [5]. In our study, those with low fish consumption (less than once a week or more) also tended to be younger (Papers II and III).

Dietary pattern analysis may capture the effects of dietary exposure that are often lost in single nutrient or food analyses. Still, the use of dietary patterns may be misleading and may hide positive effects of different foods when the dietary intake is summarized into categories or dietary patterns. Different foods or food groups may also work against each other, for example if food considered healthy is placed in a group with food considered unhealthy. An example of the latter is when fish and shellfish and the intake of meat, processed meat, mayonnaise and eggs are grouped together in a pattern, which one study did when investigating associations between major dietary patterns and the prevalence of MetS [31]. Still, the effects of higher fish consumption may be associated with a healthier lifestyle, so other factors included in a healthy lifestyle such as other healthy food and a higher level of physical activity may be confounding factors. Furthermore, the cardiac benefits of fish consumption may vary depending on how the fish is prepared (e.g., baked or fried) [163], and positive health effects may be diminished or may vanish depending on preparation, which has been observed with fried fish in studies concerning CVD [164] and DM2 [12]. Therefore, any association found may be due to either foods such as fish or to the additive effects of different foods consumed together. Today, lifestyle interventions remain the primary therapy for MetS [165], and it is therefore important to focus on lifestyle factors such as diet and foods such as fish and their possible health implications related to MetS and its components.

6 Conclusions and further research

In summary, these findings support the hypothesis that fish consumption may be associated with a lower risk of MetS, and thus a healthier metabolic profile. However, fatty and lean fish consumption may influence MetS risk differently, possibly related to age and gender.

The results from this thesis suggest that lean fish in particular may be associated with lower risk of MetS and beneficial changes in the MetS components. Today, consumption of fatty fish is highly recommended. However, the associations revealed herein suggest that lean fish consumption may also have a place in MetS prevention.

This thesis adds valuable information to the body of knowledge about fish consumption and MetS. However, it is hypothesis-generating research, and therefore, it is too early to suggest any possible recommendation in terms of increased lean fish consumption.

Our findings should be further validated using studies with other designs, especially to clarify the role of lean versus fatty fish in association with MetS risk.

In future studies, it would be of interest to investigate effects of lean fish and fatty fish on MetS separately in randomized controlled trials. This could clarify the role of lean versus fatty fish consumption in relation to MetS risk.

References

1. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr.: **Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.** *Circulation* 2009, **120:**1640-1645.
2. Laclaustra M, Corella D, Ordovas JM: **Metabolic syndrome pathophysiology: the role of adipose tissue.** *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2007, **17:**125-139.
3. Sah SK, Khatiwada S, Pandey S, Kc R, Das BK, Baral N, Lamsal M: **Association of high-sensitivity C-reactive protein and uric acid with the metabolic syndrome components.** *SpringerPlus* 2016, **5:**269.
4. Raatz SK, Silverstein JT, Jahns L, Picklo MJ: **Issues of fish consumption for cardiovascular disease risk reduction.** *Nutrients* 2013, **5:**1081-1097.
5. Strom M, Halldorsson TI, Mortensen EL, Torp-Pedersen C, Olsen SF: **Fish, n-3 fatty acids, and cardiovascular diseases in women of reproductive age: a prospective study in a large national cohort.** *Hypertension* 2012, **59:**36-43.
6. He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, Greenland P: **Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies.** *Circulation* 2004, **109:**2705-2711.
7. Li YH, Zhou CH, Pei HJ, Zhou XL, Li LH, Wu YJ, Hui RT: **Fish consumption and incidence of heart failure: a meta-analysis of prospective cohort studies.** *Chinese medical journal* 2013, **126:**942-948.
8. Chowdhury R, Stevens S, Gorman D, Pan A, Warnakula S, Chowdhury S, Ward H, Johnson L, Crowe F, Hu FB, Franco OH: **Association between fish consumption, long chain omega 3 fatty acids, and risk of cerebrovascular disease: systematic review and meta-analysis.** *BMJ* 2012, **345:**e6698.
9. Takata Y, Zhang X, Li H, Gao YT, Yang G, Gao J, Cai H, Xiang YB, Zheng W, Shu XO: **Fish intake and risks of total and cause-specific mortality in 2 population-based cohort studies of 134,296 men and women.** *American journal of epidemiology* 2013, **178:**46-57.
10. Streppel MT, Ocke MC, Boshuizen HC, Kok FJ, Kromhout D: **Long-term fish consumption and n-3 fatty acid intake in relation to (sudden) coronary heart disease death: the Zutphen study.** *Eur Heart J* 2008, **29:**2024-2030.
11. Panagiotakos DB, Zeimbekis A, Boutziouka V, Economou M, Kourlaba G, Toutouzas P, Polychronopoulos E: **Long-term fish intake is associated with better lipid profile, arterial blood pressure, and blood glucose levels in elderly people from Mediterranean islands (MEDIS epidemiological study).** *Medical science monitor : international medical journal of experimental and clinical research* 2007, **13:**CR307-312.
12. Patel PS, Sharp SJ, Luben RN, Khaw KT, Bingham SA, Wareham NJ, Forouhi NG: **Association between type of dietary fish and seafood intake and the risk of**

incident type 2 diabetes: the European prospective investigation of cancer (EPIC)-Norfolk cohort study. *Diabetes care* 2009, **32:**1857-1863.
13. Rylander C, Sandanger TM, Engeset D, Lund E: **Consumption of lean fish reduces the risk of type 2 diabetes mellitus: a prospective population based cohort study of Norwegian women.** *PloS one* 2014, **9:**e89845.
14. Pot GK, Geelen A, Majsak-Newman G, Harvey LJ, Nagengast FM, Witteman BJ, van de Meeberg PC, Hart AR, Schaafsma G, Lund EK, et al: **Increased consumption of fatty and lean fish reduces serum C-reactive protein concentrations but not inflammation markers in feces and in colonic biopsies.** *The Journal of nutrition* 2010, **140:**371-376.
15. Baik I, Abbott RD, Curb JD, Shin C: **Intake of fish and n-3 fatty acids and future risk of metabolic syndrome.** *Journal of the American Dietetic Association* 2010, **110:**1018-1026.
16. Kouki R, Schwab U, Hassinen M, Komulainen P, Heikkila H, Lakka TA, Rauramaa R: **Food consumption, nutrient intake and the risk of having metabolic syndrome: the DR's EXTRA Study.** *European journal of clinical nutrition* 2011, **65:**368-377.
17. Ruidavets JB, Bongard V, Dallongeville J, Arveiler D, Ducimetiere P, Perret B, Simon C, Amouyel P, Ferrieres J: **High consumptions of grain, fish, dairy products and combinations of these are associated with a low prevalence of metabolic syndrome.** *Journal of epidemiology and community health* 2007, **61:**810-817.
18. Robinson LE, Mazurak VC: **N-3 polyunsaturated fatty acids: relationship to inflammation in healthy adults and adults exhibiting features of metabolic syndrome.** *Lipids* 2013, **48:**319-332.
19. Robinson LE, Buchholz AC, Mazurak VC: **Inflammation, obesity, and fatty acid metabolism: influence of n-3 polyunsaturated fatty acids on factors contributing to metabolic syndrome.** *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* 2007, **32:**1008-1024.
20. Eckel RH, Grundy SM, Zimmet PZ: **The metabolic syndrome.** *Lancet* 2005, **365:**1415-1428.
21. Alberti KG, Zimmet PZ: **Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation.** *Diabetic medicine : a journal of the British Diabetic Association* 1998, **15:**539-553.
22. National Cholesterol Education Program Expert Panel on Detection E, Treatment of High Blood Cholesterol in A: **Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report.** *Circulation* 2002, **106:**3143-3421.
23. Alberti KG, Zimmet P, Shaw J: **The metabolic syndrome--a new worldwide definition.** *Lancet* 2005, **366:**1059-1062.
24. Duarte Pimentel G, Portero-McLellan KC, Maesta N, Corrente JE, Burini RC: **Accuracy of sagittal abdominal diameter as predictor of abdominal fat among Brazilian adults: a comparation with waist circumference.** *Nutr Hosp* 2010, **25:**656-661.
25. Wajchenberg BL: **Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome.** *Endocrine reviews* 2000, **21:**697-738.
26. Grundy SM: **Metabolic syndrome pandemic.** *Arteriosclerosis, thrombosis, and vascular biology* 2008, **28:**629-636.

27. Cohen A, Pieper CF, Brown AJ, Bastian LA: **Number of children and risk of metabolic syndrome in women.** *J Womens Health (Larchmt)* 2006, **15:**763-773.
28. Gunderson EP, Jacobs DR, Jr., Chiang V, Lewis CE, Feng J, Quesenberry CP, Jr., Sidney S: **Duration of lactation and incidence of the metabolic syndrome in women of reproductive age according to gestational diabetes mellitus status: a 20-Year prospective study in CARDIA (Coronary Artery Risk Development in Young Adults).** *Diabetes* 2010, **59:**495-504.
29. Torris C, Thune I, Emaus A, Finstad SE, Bye A, Furberg AS, Barrett E, Jasienska G, Ellison P, Hjartaker A: **Duration of Lactation, Maternal Metabolic Profile, and Body Composition in the Norwegian EBBA I-Study.** *Breastfeeding medicine : the official journal of the Academy of Breastfeeding Medicine* 2013, **8:**8-15.
30. Cornier MA, Dabelea D, Hernandez TL, Lindstrom RC, Steig AJ, Stob NR, Van Pelt RE, Wang H, Eckel RH: **The metabolic syndrome.** *Endocrine reviews* 2008, **29:**777-822.
31. Akter S, Nanri A, Pham NM, Kurotani K, Mizoue T: **Dietary patterns and metabolic syndrome in a Japanese working population.** *Nutrition & metabolism* 2013, **10:**30.
32. Jung HJ, Han SN, Song S, Paik HY, Baik HW, Joung H: **Association between adherence to the Korean Food Guidance System and the risk of metabolic abnormalities in Koreans.** *Nutrition research and practice* 2011, **5:**560-568.
33. Panagiotakos DB, Pitsavos C, Skoumas Y, Stefanadis C: **The association between food patterns and the metabolic syndrome using principal components analysis: The ATTICA Study.** *Journal of the American Dietetic Association* 2007, **107:**979-987; quiz 997.
34. Fonseca MJ, Gaio R, Lopes C, Santos AC: **Association between dietary patterns and metabolic syndrome in a sample of Portuguese adults.** *Nutr J* 2012, **11:**64.
35. He Y, Li Y, Lai J, Wang D, Zhang J, Fu P, Yang X, Qi L: **Dietary patterns as compared with physical activity in relation to metabolic syndrome among Chinese adults.** *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2012.
36. Alvarez Leon E, Henriquez P, Serra-Majem L: **Mediterranean diet and metabolic syndrome: a cross-sectional study in the Canary Islands.** *Public Health Nutr* 2006, **9:**1089-1098.
37. Hong S, Song Y, Lee KH, Lee HS, Lee M, Jee SH, Joung H: **A fruit and dairy dietary pattern is associated with a reduced risk of metabolic syndrome.** *Metabolism: clinical and experimental* 2012, **61:**883-890.
38. Pasalic D, Dodig S, Corovic N, Pizent A, Jurasovic J, Pavlovic M: **High prevalence of metabolic syndrome in an elderly Croatian population - a multicentre study.** *Public Health Nutr* 2011, **14:**1650-1657.
39. Hildrum B, Mykletun A, Hole T, Midthjell K, Dahl AA: **Age-specific prevalence of the metabolic syndrome defined by the International Diabetes Federation and the National Cholesterol Education Program: the Norwegian HUNT 2 study.** *BMC public health* 2007, **7:**220.
40. Cameron AJ, Boyko EJ, Sicree RA, Zimmet PZ, Soderberg S, Alberti KG, Tuomilehto J, Chitson P, Shaw JE: **Central obesity as a precursor to the metabolic syndrome in the AusDiab study and Mauritius.** *Obesity (Silver Spring)* 2008, **16:**2707-2716.
41. Rask-Madsen C, Kahn CR: **Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease.** *Arteriosclerosis, thrombosis, and vascular biology* 2012, **32:**2052-2059.
42. Bays HE: **"Sick fat," metabolic disease, and atherosclerosis.** *The American journal of medicine* 2009, **122:**S26-37.

43. Lea T: *Immunologi og immunologiske teknikker (in Norwegian).* 3. utg. edn. Bergen: Fagbokforl.; 2006.
44. The Norwegian Directorate of Health: *Utviklingen i norsk kosthold 2015 (in Norwegian).* Oslo: Helsedirektoratet; 2015.
45. The Norwegian Directorate of Health: *Anbefalinger om kosthold, ernæring og fysisk aktivitet (in Norwegian).* Helsedirektoratet; 2014.
46. Johansson L, Solvoll K, Statens råd for ernæring og fysisk a: *Norkost 1997 : landsomfattende kostholdsundersøkelse blant menn og kvinner i alderen 16-79 år (in Norwegian).* Oslo: Statens råd for ernæring og fysisk aktivitet; 1999.
47. Totland TH, Helsedirektoratet, Universitetet i O, Mattilsynet: *Norkost 3 : en landsomfattende kostholdsundersøkelse blant menn og kvinner i Norge i alderen 18-70 år, 2010-11.* Oslo: Helsedirektoratet; 2012.
48. Skåre JU, Brantsæter AL, Frøyland L, Hemre G-I, Knutsen HK, Lillegaard ITL, Torstensen B: *Benefit-risk assessment of fish and fish products in the Norwegian diet - an update (in Norwegian).* Oslo: Norwegian Scientific Committee for Food Safety (VKM); 2014.
49. Potenza MV, Mechanick JI: **The metabolic syndrome: definition, global impact, and pathophysiology.** *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition* 2009, **24:**560-577.
50. Lund EK: **Health benefits of seafood; is it just the fatty acids?** *Food chemistry* 2013, **140:**413-420.
51. *Norwegian Food Composition Database 2016.* www.matvaretabellen.no: Norwegian Food Safety Authority, The Norwegian Directorate of Health and University of Oslo.
52. Gormley T, Neumann T, Fagan J, Brunton N: **Taurine content of raw and processed fish fillets/portions.** *Zeitschrift für Lebensmittel- Untersuchung und -Forschung A* 2007, **225:**837-842.
53. *Nordic nutrition recommendations 2012 : integrating nutrition and physical activity.* 5th ed. edn. Copenhagen: Nordic Council of Ministers; 2014.
54. Moher D, Liberati A, Tetzlaff J, Altman DG: **Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement.** *BMJ* 2009, **339:**b2535.
55. Torris C, Molin M, Cvancarova Smastuen M: **Fish consumption and its possible preventive role on the development and prevalence of metabolic syndrome - a systematic review.** *Diabetol Metab Syndr* 2014, **6:**112.
56. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I: **Cohort profile: the Tromso Study.** *International journal of epidemiology* 2012, **41:**961-967.
57. Eggen AE, Mathiesen EB, Wilsgaard T, Jacobsen BK, Njolstad I: **The sixth survey of the Tromso Study (Tromso 6) in 2007-08: collaborative research in the interface between clinical medicine and epidemiology: study objectives, design, data collection procedures, and attendance in a multipurpose population-based health survey.** *Scand J Public Health* 2013, **41:**65-80.
58. Jacobsen BK, Nilsen H: **High education is associated with low fat and high fibre, beta-carotene and vitamin C – Computation of nutrient intake based on a short food frequency questionnaire in 17,265 men and women in the Tromsø Study.** *Nor Epidemiol* 2000, **10:**57-62.
59. Eggen AE, Mathiesen EB, Wilsgaard T, Jacobsen BK, Njolstad I: **Trends in cardiovascular risk factors across levels of education in a general population: is the educational gap increasing? The Tromso study 1994-2008.** *Journal of epidemiology and community health* 2014, **68:**712-719.

60. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr., et al: **Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement.** *Circulation* 2005, **112:**2735-2752.
61. Stocks T, Bjorge T, Ulmer H, Manjer J, Haggstrom C, Nagel G, Engeland A, Johansen D, Hallmans G, Selmer R, et al: **Metabolic risk score and cancer risk: pooled analysis of seven cohorts.** *International journal of epidemiology* 2015, **44:**1353-1363.
62. Kahn R, Buse J, Ferrannini E, Stern M: **The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes.** *Diabetes care* 2005, **28:**2289-2304.
63. Zahl P-H, Rognerud M, Strand BH: **Social inequality and trends in mortality among singles in Norway.** *Tidsskrift for den Norske lægeforening (in Norwegian)* 2003, **123:**1822.
64. Rothman KJ, Greenland S, Lash TL, Buehler JW, Cahill J, Glymour MM, Willett W: *Modern epidemiology.* 3rd ed. edn. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; 2008.
65. Silva IdS, World Health Organization, International Agency for Research on Cancer: *Cancer epidemiology : principles and methods.* Lyon: International Agency for Research on Cancer; 1999.
66. Krokstad S, Langhammer A, Hveem K, Holmen TL, Midthjell K, Stene TR, Bratberg G, Heggland J, Holmen J: **Cohort Profile: the HUNT Study, Norway.** *International journal of epidemiology* 2013, **42:**968-977.
67. Knudsen AK, Hotopf M, Skogen JC, Overland S, Mykletun A: **The health status of nonparticipants in a population-based health study: the Hordaland Health Study.** *American journal of epidemiology* 2010, **172:**1306-1314.
68. **The Tromsø Study** [http://www.tromsostudy.com accessed 6 May 2016]
69. Tourangeau R, Yan T: **Sensitive questions in surveys.** *Psychological bulletin* 2007, **133:**859-883.
70. Okamoto K, Ohsuka K, Shiraishi T, Hukazawa E, Wakasugi S, Furuta K: **Comparability of epidemiological information between self- and interviewer-administered questionnaires.** *Journal of clinical epidemiology* 2002, **55:**505-511.
71. Cantin J, Latour E, Ferland-Verry R, Morales Salgado S, Lambert J, Faraj M, Nigam A: **Validity and reproducibility of a food frequency questionnaire focused on the Mediterranean diet for the Quebec population.** *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2016, **26:**154-161.
72. Woods RK, Stoney RM, Ireland PD, Bailey MJ, Raven JM, Thien FC, Walters EH, Abramson MJ: **A valid food frequency questionnaire for measuring dietary fish intake.** *Asia Pacific journal of clinical nutrition* 2002, **11:**56-61.
73. Hjartaker A, Lund E, Bjerve KS: **Serum phospholipid fatty acid composition and habitual intake of marine foods registered by a semi-quantitative food frequency questionnaire.** *European journal of clinical nutrition* 1997, **51:**736-742.
74. Mina K, Fritschi L, Knuiman M: **A valid semiquantitative food frequency questionnaire to measure fish consumption.** *European journal of clinical nutrition* 2007, **61:**1023-1031.
75. Hansen-Krone IJ, Enga KF, Sudduth-Klinger JM, Mathiesen EB, Njolstad I, Wilsgaard T, Watkins S, Braekkan SK, Hansen JB: **High fish plus fish oil intake is associated with slightly reduced risk of venous thromboembolism: the Tromso Study.** *The Journal of nutrition* 2014, **144:**861-867.

76. Kahn HS, Gu Q, Bullard KM, Freedman DS, Ahluwalia N, Ogden CL: **Population distribution of the sagittal abdominal diameter (SAD) from a representative sample of US adults: comparison of SAD, waist circumference and body mass index for identifying dysglycemia.** *PloS one* 2014, **9:**e108707.
77. Martin CA, McGrath BP: **White-coat hypertension.** *Clinical and experimental pharmacology & physiology* 2014, **41:**22-29.
78. Jager KJ, Zoccali C, MacLeod A, Dekker FW: **Confounding: What it is and how to deal with it.** *Kidney International* 2008, **73:**256-260.
79. Lorente-Cebrian S, Costa AG, Navas-Carretero S, Zabala M, Martinez JA, Moreno-Aliaga MJ: **Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence.** *J Physiol Biochem* 2013, **69:**633-651.
80. Strange RC, Shipman KE, Ramachandran S: **Metabolic syndrome: A review of the role of vitamin D in mediating susceptibility and outcome.** *World journal of diabetes* 2015, **6:**896-911.
81. Slagter SN, van Vliet-Ostaptchouk JV, Vonk JM, Boezen HM, Dullaart RP, Kobold AC, Feskens EJ, van Beek AP, van der Klauw MM, Wolffenbuttel BH: **Associations between smoking, components of metabolic syndrome and lipoprotein particle size.** *BMC medicine* 2013, **11:**195.
82. Kim YS, Xun P, He K: **Fish consumption, long-chain omega-3 polyunsaturated fatty acid intake and risk of metabolic syndrome: a meta-analysis.** *Nutrients* 2015, **7:**2085-2100.
83. Zaribaf F, Falahi E, Barak F, Heidari M, Keshteli AH, Yazdannik A, Esmaillzadeh A: **Fish consumption is inversely associated with the metabolic syndrome.** *European journal of clinical nutrition* 2014, **68:**474-480.
84. Tørris C, Molin M, Cvancarova Småstuen M: **Fish consumption and its possible preventive role on the development and prevalence of metabolic syndrome - a systematic review** *Diabetology & Metabolic Syndrome* 2014, **6:112**.
85. Baik I: **Optimal cutoff points of waist circumference for the criteria of abdominal obesity: comparison with the criteria of the International Diabetes Federation.** *Circulation journal : official journal of the Japanese Circulation Society* 2009, **73:**2068-2075.
86. Kim YS, Xun P, Iribarren C, Van Horn L, Steffen L, Daviglus ML, Siscovick D, Liu K, He K: **Intake of fish and long-chain omega-3 polyunsaturated fatty acids and incidence of metabolic syndrome among American young adults: a 25-year follow-up study.** *Eur J Nutr* 2016.
87. Bogl LH, Pietilainen KH, Rissanen A, Kangas AJ, Soininen P, Rose RJ, Ala-Korpela M, Kaprio J: **Association between habitual dietary intake and lipoprotein subclass profile in healthy young adults.** *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2013.
88. Lankinen M, Schwab U, Kolehmainen M, Paananen J, Poutanen K, Mykkanen H, Seppanen-Laakso T, Gylling H, Uusitupa M, Oresic M: **Whole grain products, fish and bilberries alter glucose and lipid metabolism in a randomized, controlled trial: the Sysdimet study.** *PloS one* 2011, **6:**e22646.
89. Uusitupa M, Hermansen K, Savolainen MJ, Schwab U, Kolehmainen M, Brader L, Mortensen LS, Cloetens L, Johansson-Persson A, Onning G, et al: **Effects of an isocaloric healthy Nordic diet on insulin sensitivity, lipid profile and inflammation markers in metabolic syndrome - a randomized study (SYSDIET).** *Journal of internal medicine* 2013, **274:**52-66.

90. Telle-Hansen VH, Larsen LN, Hostmark AT, Molin M, Dahl L, Almendingen K, Ulven SM: **Daily intake of cod or salmon for 2 weeks decreases the 18:1n-9/18:0 ratio and serum triacylglycerols in healthy subjects.** *Lipids* 2012, **47:**151-160.
91. Liu X, Tao L, Cao K, Wang Z, Chen D, Guo J, Zhu H, Yang X, Wang Y, Wang J, et al: **Association of high-density lipoprotein with development of metabolic syndrome components: a five-year follow-up in adults.** *BMC public health* 2015, **15:**412.
92. Schmedes M, Aadland EK, Sundekilde UK, Jacques H, Lavigne C, Graff IE, Eng O, Holthe A, Mellgren G, Young JF, et al: **Lean-seafood intake decreases urinary markers of mitochondrial lipid and energy metabolism in healthy subjects: metabolomics results from a randomized crossover intervention study.** *Molecular nutrition & food research* 2016.
93. Thorsdottir I, Tomasson H, Gunnarsdottir I, Gisladottir E, Kiely M, Parra MD, Bandarra NM, Schaafsma G, Martinez JA: **Randomized trial of weight-loss-diets for young adults varying in fish and fish oil content.** *Int J Obes (Lond)* 2007, **31:**1560-1566.
94. Romaguera D, Norat T, Mouw T, May AM, Bamia C, Slimani N, Travier N, Besson H, Luan J, Wareham N, et al: **Adherence to the Mediterranean diet is associated with lower abdominal adiposity in European men and women.** *The Journal of nutrition* 2009, **139:**1728-1737.
95. Jakobsen MU, Due KM, Dethlefsen C, Halkjaer J, Holst C, Forouhi NG, Tjonneland A, Boeing H, Buijsse B, Palli D, et al: **Fish consumption does not prevent increase in waist circumference in European women and men.** *Br J Nutr* 2012, **108:**924-931.
96. Ramel A, Martinez JA, Kiely M, Bandarra NM, Thorsdottir I: **Moderate consumption of fatty fish reduces diastolic blood pressure in overweight and obese European young adults during energy restriction.** *Nutrition* 2010, **26:**168-174.
97. Erkkila AT, Schwab US, de Mello VD, Lappalainen T, Mussalo H, Lehto S, Kemi V, Lamberg-Allardt C, Uusitupa MI: **Effects of fatty and lean fish intake on blood pressure in subjects with coronary heart disease using multiple medications.** *Eur J Nutr* 2008, **47:**319-328.
98. El Khoury D, Anderson GH: **Recent advances in dietary proteins and lipid metabolism.** *Current opinion in lipidology* 2013, **24:**207-213.
99. Aadland EK, Lavigne C, Graff IE, Eng O, Paquette M, Holthe A, Mellgren G, Jacques H, Liaset B: **Lean-seafood intake reduces cardiovascular lipid risk factors in healthy subjects: results from a randomized controlled trial with a crossover design.** *The American journal of clinical nutrition* 2015, **102:**582-592.
100. Ouellet V, Marois J, Weisnagel SJ, Jacques H: **Dietary cod protein improves insulin sensitivity in insulin-resistant men and women: a randomized controlled trial.** *Diabetes care* 2007, **30:**2816-2821.
101. Shukla A, Bettzieche A, Hirche F, Brandsch C, Stangl GI, Eder K: **Dietary fish protein alters blood lipid concentrations and hepatic genes involved in cholesterol homeostasis in the rat model.** *Br J Nutr* 2006, **96:**674-682.
102. Lavigne C, Tremblay F, Asselin G, Jacques H, Marette A: **Prevention of skeletal muscle insulin resistance by dietary cod protein in high fat-fed rats.** *American journal of physiology Endocrinology and metabolism* 2001, **281:**E62-71.
103. Myhre JB, Loken EB, Wandel M, Andersen LF: **Differences in nutrient composition and choice of side dishes between red meat and fish dinners in Norwegian adults.** *Food Nutr Res* 2016, **60:**29555.

104. Liaset B, Hao Q, Jorgensen H, Hallenborg P, Du ZY, Ma T, Marschall HU, Kruhoffer M, Li R, Li Q, et al: **Nutritional regulation of bile acid metabolism is associated with improved pathological characteristics of the metabolic syndrome.** *The Journal of biological chemistry* 2011, **286:**28382-28395.
105. He T, Giuseppin ML: **Slow and fast dietary proteins differentially modulate postprandial metabolism.** *Int J Food Sci Nutr* 2014, **65:**386-390.
106. Tome D: **Criteria and markers for protein quality assessment - a review.** *Br J Nutr* 2012, **108 Suppl 2:**S222-229.
107. Uhe AM, Collier GR, O'Dea K: **A comparison of the effects of beef, chicken and fish protein on satiety and amino acid profiles in lean male subjects.** *The Journal of nutrition* 1992, **122:**467-472.
108. Pilon G, Ruzzin J, Rioux LE, Lavigne C, White PJ, Froyland L, Jacques H, Bryl P, Beaulieu L, Marette A: **Differential effects of various fish proteins in altering body weight, adiposity, inflammatory status, and insulin sensitivity in high-fat-fed rats.** *Metabolism: clinical and experimental* 2011, **60:**1122-1130.
109. Chamarthi B, Williams GH, Ricchiuti V, Srikumar N, Hopkins PN, Luther JM, Jeunemaitre X, Thomas A: **Inflammation and hypertension: the interplay of interleukin-6, dietary sodium, and the renin-angiotensin system in humans.** *American journal of hypertension* 2011, **24:**1143-1148.
110. Imae M, Asano T, Murakami S: **Potential role of taurine in the prevention of diabetes and metabolic syndrome.** *Amino acids* 2014, **46:**81-88.
111. Elvevoll EO, Eilertsen KE, Brox J, Dragnes BT, Falkenberg P, Olsen JO, Kirkhus B, Lamglait A, Osterud B: **Seafood diets: hypolipidemic and antiatherogenic effects of taurine and n-3 fatty acids.** *Atherosclerosis* 2008, **200:**396-402.
112. Xu YJ, Arneja AS, Tappia PS, Dhalla NS: **The potential health benefits of taurine in cardiovascular disease.** *Experimental and clinical cardiology* 2008, **13:**57-65.
113. Zhang M, Bi LF, Fang JH, Su XL, Da GL, Kuwamori T, Kagamimori S: **Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects.** *Amino acids* 2004, **26:**267-271.
114. Chen W, Guo JX, Chang P: **The effect of taurine on cholesterol metabolism.** *Molecular nutrition & food research* 2012, **56:**681-690.
115. Dragnes BT, Larsen R, Ernstsen MH, Maehre H, Elvevoll EO: **Impact of processing on the taurine content in processed seafood and their corresponding unprocessed raw materials.** *Int J Food Sci Nutr* 2009, **60:**143-152.
116. Saravanan P, Davidson NC, Schmidt EB, Calder PC: **Cardiovascular effects of marine omega-3 fatty acids.** *Lancet* 2010, **376:**540-550.
117. Holick MF: **Vitamin D deficiency.** *The New England journal of medicine* 2007, **357:**266-281.
118. Wiktorowska-Owczarek A, Berezinska M, Nowak JZ: **PUFAs: Structures, Metabolism and Functions.** *Advances in clinical and experimental medicine : official organ Wroclaw Medical University* 2015, **24:**931-941.
119. Galarraga B, Ho M, Youssef HM, Hill A, McMahon H, Hall C, Ogston S, Nuki G, Belch JJ: **Cod liver oil (n-3 fatty acids) as an non-steroidal anti-inflammatory drug sparing agent in rheumatoid arthritis.** *Rheumatology (Oxford, England)* 2008, **47:**665-669.
120. Calder PC: **n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases.** *The American journal of clinical nutrition* 2006, **83:**1505S-1519S.
121. Davidson MH: **Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids.** *The American journal of cardiology* 2006, **98:**27i-33i.

122. Dunn SL, Siu W, Freund J, Boutcher SH: **The effect of a lifestyle intervention on metabolic health in young women.** *Diabetes, metabolic syndrome and obesity : targets and therapy* 2014, **7:**437-444.
123. Lee TC, Ivester P, Hester AG, Sergeant S, Case LD, Morgan T, Kouba EO, Chilton FH: **The impact of polyunsaturated fatty acid-based dietary supplements on disease biomarkers in a metabolic syndrome/diabetes population.** *Lipids in health and disease* 2014, **13:**196.
124. Lewis A, Lookinland S, Beckstrand RL, Tiedeman ME: **Treatment of hypertriglyceridemia with omega-3 fatty acids: a systematic review.** *Journal of the American Academy of Nurse Practitioners* 2004, **16:**384-395.
125. Yanai H, Hamasaki H, Katsuyama H, Adachi H, Moriyama S, Sako A: **Effects of intake of fish or fish oils on the development of diabetes.** *Journal of clinical medicine research* 2015, **7:**8-12.
126. Pedersen MH, Molgaard C, Hellgren LI, Lauritzen L: **Effects of fish oil supplementation on markers of the metabolic syndrome.** *The Journal of pediatrics* 2010, **157:**395-400, 400 e391.
127. Cicero AF, Derosa G, Di Gregori V, Bove M, Gaddi AV, Borghi C: **Omega 3 polyunsaturated fatty acids supplementation and blood pressure levels in hypertriglyceridemic patients with untreated normal-high blood pressure and with or without metabolic syndrome: a retrospective study.** *Clinical and experimental hypertension* 2010, **32:**137-144.
128. Visioli F, Rise P, Barassi MC, Marangoni F, Galli C: **Dietary intake of fish vs. formulations leads to higher plasma concentrations of n-3 fatty acids.** *Lipids* 2003, **38:**415-418.
129. Ebbesson SO, Tejero ME, Nobmann ED, Lopez-Alvarenga JC, Ebbesson L, Romenesko T, Carter EA, Resnick HE, Devereux RB, MacCluer JW, et al: **Fatty acid consumption and metabolic syndrome components: the GOCADAN study.** *Journal of the cardiometabolic syndrome* 2007, **2:**244-249.
130. Martinez-Fernandez L, Laiglesia LM, Huerta AE, Martinez JA, Moreno-Aliaga MJ: **Omega-3 fatty acids and adipose tissue function in obesity and metabolic syndrome.** *Prostaglandins & other lipid mediators* 2015, **121:**24-41.
131. Ma T, Liaset B, Hao Q, Petersen RK, Fjaere E, Ngo HT, Lillefosse HH, Ringholm S, Sonne SB, Treebak JT, et al: **Sucrose counteracts the anti-inflammatory effect of fish oil in adipose tissue and increases obesity development in mice.** *PloS one* 2011, **6:**e21647.
132. Dahl L, Johansson L, Julshamn K, Meltzer HM: **The iodine content of Norwegian foods and diets.** *Public Health Nutr* 2004, **7:**569-576.
133. World Health Organization, Food and Agriculture Organization of the United Nations: *Vitamin & Mineral Requirements in Human Nutrition.* Geneva; 2004.
134. Ayturk S, Gursoy A, Kut A, Anil C, Nar A, Tutuncu NB: **Metabolic syndrome and its components are associated with increased thyroid volume and nodule prevalence in a mild-to-moderate iodine-deficient area.** *European journal of endocrinology / European Federation of Endocrine Societies* 2009, **161:**599-605.
135. Lecube A, Zafon C, Gromaz A, Fort J, Caubet E, Baena J, Tortosa F: **Iodine Deficiency Is Higher in Morbid Obesity in Comparison with Late After Bariatric Surgery and Non-obese Women.** *The Journal of Metabolic Surgery and Allied Care* 2015, **25:**85-89.
136. Kim YN, Kim YA, Yang AR, Lee BH: **Relationship between Blood Mercury Level and Risk of Cardiovascular Diseases: Results from the Fourth Korea National**

Health and Nutrition Examination Survey (KNHANES IV) 2008-2009. *Preventive nutrition and food science* 2014, **19:**333-342.
137. Park K, Seo E: **Association between Toenail Mercury and Metabolic Syndrome Is Modified by Selenium.** *Nutrients* 2016, **8**.
138. Taylor KW, Novak RF, Anderson HA, Birnbaum LS, Blystone C, Devito M, Jacobs D, Kohrle J, Lee DH, Rylander L, et al: **Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: a national toxicology program workshop review.** *Environmental health perspectives* 2013, **121:**774-783.
139. Valera B, Ayotte P, Poirier P, Dewailly E: **Associations between plasma persistent organic pollutant levels and blood pressure in Inuit adults from Nunavik.** *Environment international* 2013, **59:**282-289.
140. Park SH, Lim JE, Park H, Jee SH: **Body burden of persistent organic pollutants on hypertension: a meta-analysis.** *Environmental science and pollution research international* 2016.
141. Ibrahim MM, Fjære E, Lock E-J, Naville D, Amlund H, Meugnier E, Le Magueresse Battistoni B, Frøyland L, Madsen L, Jessen N, et al: **Chronic Consumption of Farmed Salmon Containing Persistent Organic Pollutants Causes Insulin Resistance and Obesity in Mice.** *PloS one* 2011, **6:**e25170.
142. Myrmel LS, Fjaere E, Midtbo LK, Bernhard A, Petersen RK, Sonne SB, Mortensen A, Hao Q, Brattelid T, Liaset B, et al: **Macronutrient composition determines accumulation of persistent organic pollutants from dietary exposure in adipose tissue of mice.** *J Nutr Biochem* 2016, **27:**307-316.
143. Guarner-Lans V, Rubio-Ruiz ME, Perez-Torres I, Banos de MacCarthy G: **Relation of aging and sex hormones to metabolic syndrome and cardiovascular disease.** *Experimental gerontology* 2011, **46:**517-523.
144. Karastergiou K, Smith SR, Greenberg AS, Fried SK: **Sex differences in human adipose tissues - the biology of pear shape.** *Biology of sex differences* 2012, **3:**13.
145. Shi H, Seeley RJ, Clegg DJ: **Sexual Differences in the Control of Energy Homeostasis.** *Frontiers in neuroendocrinology* 2009, **30:**396-404.
146. Alemany M: **Do the interactions between glucocorticoids and sex hormones regulate the development of the metabolic syndrome?** *Frontiers in endocrinology* 2012, **3:**27.
147. Stuebe AM, Rich-Edwards JW: **The reset hypothesis: lactation and maternal metabolism.** *Am J Perinatol* 2009, **26:**81-88.
148. Torris C, Thune I, Emaus A, Finstad SE, Bye A, Furberg AS, Barrett E, Jasienska G, Ellison P, Hjartaker A: **Duration of lactation, maternal metabolic profile, and body composition in the Norwegian EBBA I-study.** *Breastfeeding medicine : the official journal of the Academy of Breastfeeding Medicine* 2013, **8:**8-15.
149. Brown LM, Gent L, Davis K, Clegg DJ: **Metabolic impact of sex hormones on obesity.** *Brain research* 2010, **1350:**77-85.
150. Baik I, Lee M, Jun NR, Lee JY, Shin C: **A healthy dietary pattern consisting of a variety of food choices is inversely associated with the development of metabolic syndrome.** *Nutrition research and practice* 2013, **7:**233-241.
151. Di Daniele N, Petramala L, Di Renzo L, Sarlo F, Della Rocca DG, Rizzo M, Fondacaro V, Iacopino L, Pepine CJ, De Lorenzo A: **Body composition changes and cardiometabolic benefits of a balanced Italian Mediterranean Diet in obese patients with metabolic syndrome.** *Acta diabetologica* 2013, **50:**409-416.
152. Kesse-Guyot E, Ahluwalia N, Lassale C, Hercberg S, Fezeu L, Lairon D: **Adherence to Mediterranean diet reduces the risk of metabolic syndrome: A 6-year**

prospective study. *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2013, **23:**677-683.
153. Babio N, Bullo M, Salas-Salvado J: **Mediterranean diet and metabolic syndrome: the evidence.** *Public Health Nutr* 2009, **12:**1607-1617.
154. Cho YA, Kim J, Cho ER, Shin A: **Dietary patterns and the prevalence of metabolic syndrome in Korean women.** *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2011, **21:**893-900.
155. Kim J, Jo I: **Grains, vegetables, and fish dietary pattern is inversely associated with the risk of metabolic syndrome in South korean adults.** *Journal of the American Dietetic Association* 2011, **111:**1141-1149.
156. Williams DE, Prevost AT, Whichelow MJ, Cox BD, Day NE, Wareham NJ: **A cross-sectional study of dietary patterns with glucose intolerance and other features of the metabolic syndrome.** *Br J Nutr* 2000, **83:**257-266.
157. Lutsey PL, Steffen LM, Stevens J: **Dietary intake and the development of the metabolic syndrome: the Atherosclerosis Risk in Communities study.** *Circulation* 2008, **117:**754-761.
158. Amini M, Esmaillzadeh A, Shafaeizadeh S, Behrooz J, Zare M: **Relationship between major dietary patterns and metabolic syndrome among individuals with impaired glucose tolerance.** *Nutrition* 2010, **26:**986-992.
159. DiBello JR, McGarvey ST, Kraft P, Goldberg R, Campos H, Quested C, Laumoli TS, Baylin A: **Dietary patterns are associated with metabolic syndrome in adult Samoans.** *The Journal of nutrition* 2009, **139:**1933-1943.
160. Heidemann C, Scheidt-Nave C, Richter A, Mensink GB: **Dietary patterns are associated with cardiometabolic risk factors in a representative study population of German adults.** *Br J Nutr* 2011, **106:**1253-1262.
161. Min C, Noh H, Kang YS, Sim HJ, Baik HW, Song WO, Yoon J, Park YH, Joung H: **Skipping breakfast is associated with diet quality and metabolic syndrome risk factors of adults.** *Nutrition research and practice* 2011, **5:**455-463.
162. Song Y, Joung H: **A traditional Korean dietary pattern and metabolic syndrome abnormalities.** *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2012, **22:**456-462.
163. Mozaffarian D, Lemaitre RN, Kuller LH, Burke GL, Tracy RP, Siscovick DS: **Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study.** *Circulation* 2003, **107:**1372-1377.
164. Mozaffarian D, Lemaitre RN, Kuller LH, Burke GL, Tracy RP, Siscovick DS, Cardiovascular Health S: **Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study.** *Circulation* 2003, **107:**1372-1377.
165. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ: **The metabolic syndrome.** *Lancet* 2010, **375:**181-183.