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Fish consumption in relation to type 2 diabetes and cardiovascular complications

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Type 2 diabetes is a major public health threat globally, associated with severe medical complications such as cardiovascular diseases and damage to kidneys, eyes, and nerves, as well as with premature death. Dietary factors are of importance in both primary prevention and disease management. Fish consumption is of interest given the observed benefits on several cardiometabolic risk factors. Such benefits have largely been attributed to the content of long-chain omega-3 fatty acids, but may also be related to a wider range of nutrients. On the negative side, there is also the potential of adverse effects by contaminants present in some types of fish.

This thesis includes four papers in which we aimed to: 1) summarize results from prospective studies on the associations of fish consumption and intake of long-chain omega-3 fatty acids with risk of type 2 diabetes; 2) evaluate the validity of food frequency questionnaire (FFQ)-based estimates of these exposures; 3) examine the associations of total and specific types of fish with risk of type 2 diabetes, taking environmental contaminants into account; and 4) examine the associations of total and specific types of fish with risk of myocardial infarction (MI), stroke, and mortality among people with diagnosed type 2 diabetes.

Our meta-analysis showed that the accumulated observational evidence on fish consumption and long-chain omega-3 fatty acid intake in relation to type 2 diabetes risk is heterogeneous across regions, with an increased risk reported in studies conducted in the US, no association reported in European studies, and lower risk reported in Asian/Australian studies.

The three latter papers are based on data from two large Swedish population-based cohorts of nearly 90,000 women and men, whose dietary habits were assessed via FFQs in 1997. We showed that the estimates of long-chain omega-3 fatty acid intake correlated with adipose tissue content of these fatty acids in a subsample of 239 women, supporting their validity. Participants were followed for the outcomes of interest through December 2012, by linkage to nationwide registers. In men, total fish consumption was not associated with type 2 diabetes in the primary analysis. Taking dietary exposure to polychlorinated biphenyls (PCBs) and methylmercury (MeHg) into account, there was however a suggestion of an inverse association. High consumption of shellfish and fried fish was associated with higher risk of type 2 diabetes. Among women and men with type 2 diabetes before baseline, fish consumption was inversely associated with MI, while there was no support for an association with stroke. The results for total and coronary heart disease (CHD)-related mortality were inconclusive with some suggestion of lower risk associated with moderate fish consumption.

Overall, the results presented in this thesis do not challenge the current recommendations on regular fish consumption. General advice may however be too imprecise, and more specific recommendations on fish species and preparation methods may be warranted.

POPULÄRVETENSKAPLIG SAMMANFATTNING

I den här avhandlingen undersöks sambanden mellan fiskkonsumtion och uppkomst av typ 2-diabetes samt några av de komplikationer som är kopplade till sjukdomen.

Typ 2-diabetes är ett omfattande folkhälsoproblem över hela världen. Förekomsten har ökat kraftigt under de senaste decennierna, och ökningen förväntas fortsätta. Vid diabetes är blodsockerhalten förhöjd på grund av brist på hormonet insulin som behövs för att sockret ska tas upp i cellerna. Typ 2-diabetes är den vanligaste formen och debuterar oftast vid vuxen ålder. Vanligtvis kommer den smygande med otydliga tidiga symptom, vilket gör att det kan dröja flera år innan sjukdomen upptäcks. Vid typ 2-diabetes kan kroppen fortfarande tillverka insulin, men på grund av *insulinresistens* svarar cellerna inte som förväntat och insulinet räcker därför inte för att hålla blodsockret under kontroll. De höga sockernivåerna kan leda till skador på blodkärlen och därigenom till ett flertal följdsjukdomar. Påverkan på ögon, njurar och nerver kan i förlängningen ge allvarliga problem som synförsämringar, nedsatt känsel och fotsår, samt njursvikt. Personer med diabetes löper även en flerfalt förhöjd risk att utveckla hjärtkärlsjukdomar som hjärtinfarkt och stroke, som också är de främsta orsakerna till förhöjd dödlighet i den här gruppen.

Sjukdomen beror delvis på ärftliga faktorer, men det är även väl belagt att levnadsvanor är av stor betydelse. Det finns en stark koppling till övervikt och fysisk inaktivitet, eftersom dessa faktorer bidrar till insulinresistens. På senare tid har mer och mer forskning undersökt betydelsen av specifika kostfaktorer, men mycket återstår att klarlägga. För de som redan insjuknat i diabetes är goda kostvanor av stor betydelse för att hålla blodsockernivåerna under kontroll och förebygga uppkomsten av komplikationer.

Fisk är ett högintressant livsmedel, eftersom det har kopplats till fördelaktiga effekter på riskfaktorer som till exempel blodtryck och blodfetter. Man har också sett att de som äter mycket fisk löper lägre risk att insjukna i hjärtkärlsjukdom jämfört med de som äter lite fisk. Man tror att detta till stor del beror på fiskens innehåll av omega-3 fettsyror, men den lägre risken skulle även kunna kopplas till andra viktiga näringsämnen. Ett orosmoment är dock att vissa typer av fisk innehåller höga halter av miljöföroreningar som kan ha negativ inverkan. Tidigare studier som har undersökt sambandet mellan fiskkonsumtion och uppkomst av typ-2 diabetes har visat tvetydiga resultat. Väldigt få studier har hittills genomförts för att undersöka fiskens betydelse för uppkomst av hjärtkärlsjukdomar bland personer med diabetes vid studiens start. Syftet med denna avhandling var därför att undersöka dessa samband vidare.

De fyra ingående delarbetena syftade till att: 1) genom en metaanalys sammanfatta resultat från tidigare uppföljningsstudier på fiskkonsumtion och intag omega-3 fettsyror i relation till uppkomst av typ 2-diabetes; 2) utvärdera hur väl rapportering av fiskkonsumtion och intag av

omega-3 fettsyror som gjorts med hjälp av kostenkäter stämmer överens med uppmätta nivåer av omega-3 fettsyror i fettvävnad; 3) undersöka sambandet mellan fiskkonsumtion och uppkomst av typ 2-diabetes, med hänsyn tagen även till miljögifter i fisk; och 4) undersöka sambandet mellan fiskkonsumtion och risk för hjärtinfarkt, stroke och dödlighet bland personer med typ 2-diabetes från studiens start.

Vår metaanalys i det **första delarbetet** visade att resultaten från hittills publicerad forskning på fiskkonsumtion och intag av omega-3 fettsyror i förhållande till uppkomst av typ 2-diabetes i stor utsträckning skiljer sig åt mellan olika delar av världen. Sammanfattningen av studier från USA tyder på förhöjd risk, medan europeiska studier inte sett något samband och studier från Asien och Australien tyder på lägre risk. Vad detta beror på återstår att studera, men skulle rimligtvis kunna kopplas till skillnader i konsumtionsmönster, tillagningsmetoder, vilken typ av fisk man äter och möjligtvis skillnader i fiskens innehåll av miljöföroreningar.

För de senare tre delarbetena användes data från två stora svenska studier på kvinnor och män som rapporterade sina kostvanor via detaljerade enkäter under hösten 1997. I det **andra delarbetet** kunde vi påvisa att enkätsvaren på ett adekvat sätt speglar det faktiska intaget. Detta gjordes genom att jämföra det självrapporterade intaget av fisk och vår enkätbaserade beräkning av omega-3 fettsyreintag mot uppmätta nivåer av dessa fettsyror i fettvävnaden hos en mindre grupp av kvinnorna i studien.

Via nationella register identifierade vi studiedeltagare som insjuknat i typ 2-diabetes, hjärtinfarkt eller stroke, samt de som avlidit fram till och med 31 december 2012. Genom statistiska modeller som tar hänsyn till andra kost- och levnadsvanor beräknades sedan hur risken för dessa utfall varierade med den rapporterade fiskkonsumtionen.

Våra huvudresultat från det **tredje delarbetet** visade att fiskkonsumtion inte var kopplad till uppkomst av typ 2-diabetes. Vi kunde se en antydan till lägre risk efter att vi med statistiska verktyg bortsett från miljöföroreningar i fisken (PCB och kvicksilver). Detta är dock en teoretisk modell, och mer forskning är nödvändig för att utreda betydelsen av dessa föroreningar. Risken för typ 2-diabetes var högre bland de deltagare som rapporterade hög konsumtion av skaldjur och stekt fisk.

I det **fjärde delarbetet** utgick vi från deltagare som hade typ 2-diabetes redan vid studiens start. Fiskkonsumtion var i denna grupp kopplad till lägre risk för hjärtinfarkt, medan vi inte fann något stöd för en koppling till stroke. Resultaten för dödlighet var inte tillräckligt tydliga för att dra några säkra slutsatser, men antydde fördelar förknippade med måttlig konsumtion av fisk.

Sammanfattningsvis stödjer resultaten från denna avhandling de befintliga kostråden om regelbunden fiskkonsumtion. Mer specifika rekommendationer om olika fiskarter, ursprung och tillagningsmetoder kan dock vara motiverade.

LIST OF PUBLICATIONS

I. **Wallin A**, Di Giuseppe D, Orsini N, Patel PS, Forouhi NG, Wolk A
Fish consumption, dietary long-chain n-3 fatty acids, and risk of type 2 diabetes: systematic review and meta-analysis of prospective studies
Diabetes Care 2012;35:918-29

II. **Wallin A**, Di Giuseppe D, Burgaz A, Håkansson N, Cederholm T, Michaëlsson K, Wolk A
Validity of food frequency questionnaire-based estimates of long-term long-chain n-3 polyunsaturated fatty acid intake
Eur J Nutr 2014;53:549-55

III. **Wallin A**, Di Giuseppe D, Orsini N, Åkesson A, Forouhi NG, Wolk A
Fish consumption and frying of fish in relation to type 2 diabetes incidence: a prospective cohort study of Swedish men
Eur J Nutr. 2015 Dec 21. [Epub ahead of print]

IV. **Wallin A**, Orsini N, Forouhi NG, Wolk A
Fish consumption in relation to myocardial infarction, stroke and mortality among women and men with type 2 diabetes: a prospective cohort study
Submitted manuscript

These publications are referred to in the text as **paper I – IV**, and reproduced in full at the end of the thesis.

OTHER PUBLICATIONS

Wallin A, Orsini N, Wolk A
Red and processed meat consumption and risk of ovarian cancer: a dose–response meta-analysis of prospective studies
Br J Cancer 2011;104:1196-201

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Diabetologia 2016 Mar 18. [Epub ahead of print]

Kaluza J, Harris H, **Wallin A**, Lindén A, Wolk A
Dietary fiber intake and risk of chronic obstructive pulmonary disease: a prospective cohort study of men
Submitted manuscript

These publications are not discussed in the thesis.

CONTENTS

LIST OF ABBREVIATIONS

AHEI	Alternate Healthy Eating Index
ALA	Alpha-linolenic acid
BMI	Body mass index
CHD	Coronary heart disease
CI	Confidence interval
COSM	Cohort of Swedish Men
DASH	Dietary Approaches to Stop Hypertension
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DXA	Dual-energy X-ray absorptiometry
EPA	Eicosapentaenoic acid
FFQ	Food frequency questionnaire
HbA_{1c}	Glycated hemoglobin
HDL	High density lipoprotein
HR	Hazard ratio
ICD	International Classification of Diseases
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
LADA	Latent autoimmune diabetes in adults
LDL	Low density lipoprotein
MeHg	Methylmercury
MET	Metabolic equivalent
MI	Myocardial infarction
PCB	Polychlorinated biphenyl
RR	Relative risk
SMC	Swedish Mammography Cohort
SMC-C	Swedish Mammography Cohort Clinical
WHO	World Health Organization

1 INTRODUCTION

This thesis focuses on the associations of fish consumption with risk of incident type 2 diabetes and some of its cardiovascular complications, including myocardial infarction (MI) and stroke, as well as mortality.

Type 2 diabetes is one of the world's major public health problems, with a prevalence that has been rapidly increasing over the past decades and is expected to continue to do so in the coming years.[1] It is a severe disease associated with serious medical complications and premature death. The excess blood glucose that characterizes diabetes can directly or indirectly cause damage to the cardiovascular system, kidneys, nerves, and eyes, and thus impose a great personal burden for affected individuals as well as a substantial economic burden on health care systems.

Lifestyle factors, including diet, are key components in primary prevention as well as in disease management and prevention of complications. However, gaps in the scientific evidence for dietary recommendations of specific foods still remain. The role of fish consumption in development and progression of type 2 diabetes is an area in need of clarification. Although fish has been associated with beneficial effects on risk factors associated with diabetes,[2] as well as on cardiovascular disease development in the general population,[3-5] studies on type 2 diabetes incidence have provided largely heterogonous results,[6-19] and studies on cardiovascular disease outcomes specifically in populations with diabetes are scarce.[20-22]

Fish is generally a highly nutritious food, containing a number of important nutrients that may be hard to obtain in sufficient amounts from other food sources. Various fish species however differ in nutritional composition, and factors such as preparation method may further influence the benefits of consumption. Fish is also a major source of dietary exposure to some environmental contaminants, such as polychlorinated biphenyls (PCBs) and methylmercury (MeHg). Thus, consideration of several aspects is of importance to enhance the understanding of the role of fish consumption and eventually allow for development of more specific dietary recommendations.

2 BACKGROUND

2.1 TYPE 2 DIABETES

Diabetes mellitus is a group of metabolic diseases, all characterized by hyperglycemia resulting from insulin deficiency. Type 2 diabetes is the most common form, accounting for about 90% of all diabetes, and a major public health problem throughout the world.[1] To distinguish it from type 1 diabetes, which is often diagnosed in childhood, type 2 diabetes has commonly been referred to as *adult-onset diabetes*. However, with growing prevalence it is increasingly seen in younger age groups.[23] Another historical name aiming to distinguish it from type 1 diabetes, which is invariably treated with insulin, was *non-insulin dependent diabetes*. However, although it is often initially managed without insulin treatment, many type 2 diabetes patients come to rely on insulin therapy as the disease progresses.[24] The difference between type 1 and type 2 diabetes lies in the underlying pathophysiological defects. Whereas type 1 diabetes is an autoimmune disease that causes destruction of insulin producing cells, the insulin deficiency in type 2 diabetes is relative and progresses as the body can no longer compensate for an increased insulin demand.[25]

2.1.1 Descriptive epidemiology

According to prevalence estimates from the International Diabetes Federation (IDF) for 2015, 415 million people worldwide (8.8% of the adult population) are living with diabetes. This estimate includes 193 million undiagnosed.[1] The prevalence has been increasing continuously over the past decades, and is predicted to rise to 642 million by 2040.[1]

In large parts of the world, the prevalence has increased dramatically alongside an economic development that has driven rapid changes in age distribution as well as in lifestyle behaviors.[1] The highest age-adjusted percentage prevalence is presently found in the North America and Caribbean Region (**Figure 2.1**). The largest absolute number is found in the highly populous Western Pacific Region (153 million people).[1]

In Sweden, diabetes prevalence among adults was estimated to 4.2% based on self-reports from the latest Living Conditions Surveys (ULF/SILC) in 2012–2013.[26] The IDF estimation, including undiagnosed cases, is a total prevalence of 6.3% of adults.[1] An increase in prevalence has been seen over the past decades, although less drastic than in many other parts of the world.[27-29] The incidence however appears to have leveled off, and the rise in prevalence is mainly ascribed to changes in the age distribution and to improved survival among people with diabetes.[27-29] Given the increase in overweight and obesity in Sweden over the past decades, not the least in children and young adults, it is possible that an increase

in diabetes incidence is yet to manifest itself.[30] However, also with a stable incidence, or even some decline, changes in age structure and survival will result in a continued rise in prevalence, with a major impact on public health.[27]

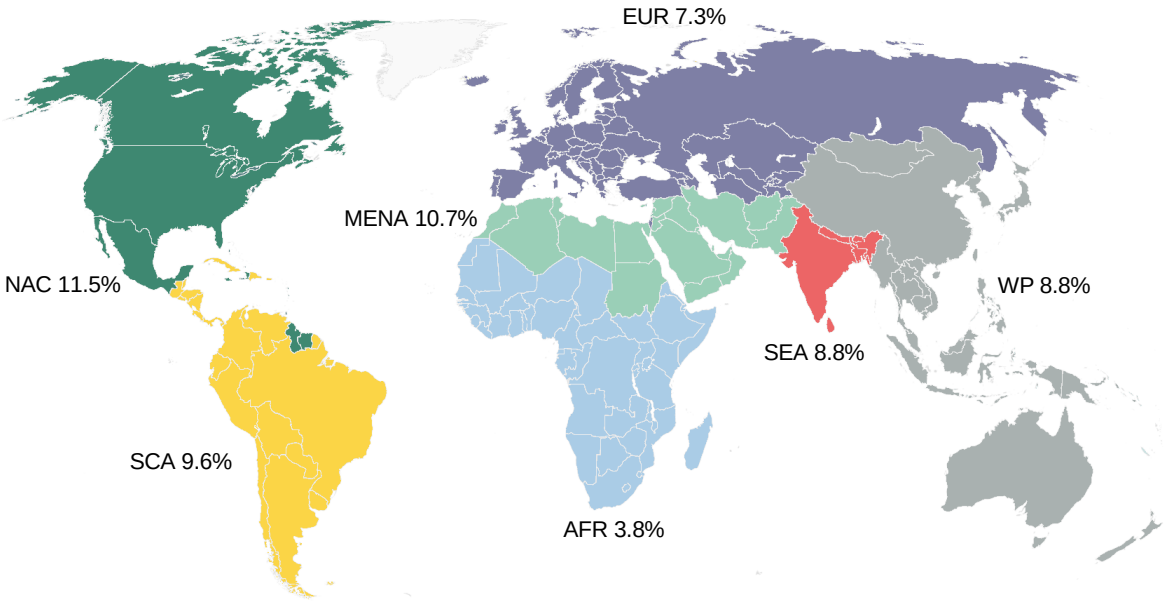


Figure 2.1 Age-adjusted prevalence of diabetes among adults (20–79 years), by International Diabetes Federation region. *AFR*, Africa; *EUR*, Europe; *MENA*, Middle East and North Africa; *NAC*, North America and Caribbean; *SCA*, South and Central America; *SEA*, South East Asia; *WP*, Western Pacific. Data source: International Diabetes Federation[1]

2.1.2 Pathophysiology

The two core pathophysiological features in type 2 diabetes are insulin resistance and pancreatic β-cell dysfunction. Blood glucose levels deteriorate only if insulin resistance is accompanied by an impaired capacity to enhance insulin secretion.[31] The onset of the disease is gradual and impairment of insulin action and β-cell function have generally progressed over several years before type 2 diabetes can be diagnosed.[32]

The manifestations of inadequate insulin response in the main insulin-sensitive tissues – skeletal muscle, liver, and adipose tissue – contribute to hyperglycemia in different ways. Uptake in skeletal muscle is a key mechanism for postprandial glucose disposal, and impaired insulin-mediated transport into the cells contributes to increasing blood levels.[31] In the liver, glucose production is increased due to impaired insulin-induced suppression.[31] In adipocytes, insulin-mediated regulation of lipolysis is disturbed, leading to elevated levels of circulating free fatty acids.[33] Chronic exposure to excess free fatty acids aggravates hyperglycemia by stimulating hepatic gluconeogenesis, inducing insulin resistance and impairing insulin secretion; a phenomenon referred to as lipotoxicity.[31, 33] Moreover, hyperglycemia itself accelerates the disease progression by impairing both insulin action and insulin secretion; a vicious cycle known as glucotoxicity.[31]

2.1.3 Diagnosis and definitions

The World Health Organization (WHO) diagnostic criteria for diabetes are fasting plasma glucose ≥7.0 mmol/L and/or plasma glucose ≥11.1 mmol/L two hours after an oral glucose load of 75 grams.[34] Measurement of glycated hemoglobin (HbA_{1c}), which reflects average blood glucose levels over the previous 8–12 weeks, is an alternative diagnostic test. This method may be more practical as is does not require a fasting sample. An HbA_{1c} of 48 mmol/mol (6.5%) is the recommended cut point for diagnosis.[35]

Diabetes type is assigned largely based on the circumstances at the time of diagnosis, and not all patients are easily classified.[36] The majority of diabetes cases fall into the two broad categories of type 1 or type 2. There are also other types, such as gestational diabetes, monogenic forms, and secondary diabetes due to pancreatic damage.[36] A type that is often misinterpreted as type 2, but shares the autoimmune characteristics of type 1, is latent autoimmune diabetes in adults (LADA).[37]

Because of the slow onset and non-specific initial symptoms, type 2 diabetes often remains undiagnosed for several years.[25] It is not uncommon that severe complications have emerged already before diagnosis.[1] The proportion of undiagnosed is largest in low- and middle-income countries. In Sweden it is estimated that about 38% of all adults with diabetes are unaware of their disease.[1]

2.1.3.1 Prediabetes

The hyperglycemia in type 2 diabetes develops progressively via a stage commonly referred to as prediabetes. The definition of prediabetes includes impaired fasting glucose (IFG; fasting plasma glucose of 6.1–6.9 mmol/L[34] or 5.6–6.9 mmol/L[25]) and impaired glucose tolerance (IGT; two-hour postload plasma glucose of 7.8–11.0 mmol/L[34]). People with prediabetes are at high risk of developing type 2 diabetes, but lifestyle or pharmacological interventions in this high-risk group have been shown to be effective to prevent progression.[38]

2.1.3.2 Metabolic syndrome

Prediabetes commonly clusters with other cardiometabolic risk factors which together are referred to as the metabolic syndrome. Several definitions of the metabolic syndrome exist, including some combination of elevated fasting glucose or glucose intolerance, abdominal obesity, high triglyceride levels, low high density lipoprotein (HDL) cholesterol, and hypertension.[39] This concept identifies a patient group at high risk of both type 2 diabetes and cardiovascular disease.

2.1.4 Complications

Type 2 diabetes is associated with a number of medical complications due to damages caused by long-term elevated blood glucose and related metabolic disturbances. These affect the heart and blood vessels, eyes, kidneys, and nerves, and are typically classified into microvascular and macrovascular complications.[40]

2.1.4.1 Microvascular complications

Microvascular complications refer to damage to the small blood vessels, and include complications in the eyes, nerves, and kidneys. In most high-income countries, diabetes is a leading cause of blindness, lower-limb amputations, and kidney failure.[1] Development and severity of these complications is strongly associated with the magnitude and duration of hyperglycemia.[40]

Damage to the retina of the eye affects most individuals with diabetes to some extent, and often begin to develop long before the diabetes diagnosis.[40] If severe, it can cause visual impairment and even blindness, but it can become quite advanced before the vision is affected.[1] Nerve damage most commonly affects peripheral nerves. Sensation loss can cause injuries to go unnoticed and contribute to foot problems for many patients. Poor circulation and elevated susceptibility to infection may further contribute to ulceration, which in serious cases requires amputation.[1] Nerve damage can occur also in other organ systems and cause problems such as erectile dysfunction, bladder dysfunction, and problems with digestion.[40] Signs of kidney dysfunction at the level of microalbuminuria are common among type 2 diabetes patients. Without proper intervention it may progress to more severe kidney disease and kidney failure.[40]

2.1.4.2 Macrovascular complications

Macrovascular complications refer to complications of the larger blood vessels, contributing to atherosclerosis and increased risk of angina, myocardial infarction (MI), stroke, congestive heart failure, and peripheral artery disease.[1] The risk of developing cardiovascular disease is several times higher in people with type 2 diabetes compared to the general population, with a greater risk increase in women than in men.[41] This group of diseases constitute the primary cause of death among people with diabetes, as well as the main component of diabetes-related health care expenditures.[40]

Type 2 diabetes is commonly accompanied by a cluster of traditional cardiovascular risk factors, such as elevated low density lipoprotein (LDL) cholesterol levels, low HDL cholesterol levels, and high blood pressure. The cardiovascular risk among people with diabetes can however not be explained by these factors alone, but is further added to by the effects of hyperglycemia and insulin resistance. These effects include aggravated dyslipidemia and hypertension, overproduction of reactive oxygen species, low-grade inflammation, impaired endothelial function, and thrombotic abnormalities.[41]

2.1.5 Management

The main treatment goal in type 2 diabetes is to control blood glucose levels in order to prevent and manage its complications. Management of coexisting conditions such as hypertension and dyslipidemia is also of major importance.[42, 43] Most patients are initially treated with oral blood glucose-lowering drugs in combination with counseling on healthy diet and physical activity. As the disease progresses, many patients come to depend on insulin treatment.[24, 42]

Recommendations for dietary management of diabetes from the American Diabetes Association,[44] as well as guidelines from the Swedish National Board of Health and Welfare,[45] recognize that a main focus for many patients is to control overall energy intake. Emphasis is put on individualization and flexibility to allow long-term adherence to the dietary plan. Monitoring carbohydrate intake is central for glycemic control, but there is no support for very low carbohydrate diets. Instead, the focus should be on quality rather than quantity. A 2013 meta-analysis of twenty randomized controlled trials of dietary interventions in type 2 diabetes concluded that a variety of diets, such as low-carbohydrate, low glycemic index, Mediterranean, and high-protein diets, may be effective for improving glycemic control, lipid profile, and body weight.[46] Evidence from studies on dietary factors in relation to major cardiovascular events and mortality in people with diabetes is more sparse, and recommendations on specific foods and nutrients are to some extent based on what is known about healthy eating habits for the general population.[44, 45, 47]

2.1.6 Risk factors

Although the causes of type 2 diabetes are not completely understood, several risk factors of importance have been identified. Both genetic susceptibility and triggering environmental risk factors, as well as interactions between the two, contribute to disease development.[48]

2.1.6.1 Non-modifiable factors

Family history has long been recognized as an important risk factor, with first-degree relatives being at two- to sixfold higher risk of developing type 2 diabetes.[49] Twin studies have further supported a strong genetic link, with an estimated heritability of 40–80%.[50] More recently, the advancements of genome-wide association methodology have rapidly increased the number of identified genetic variants associated with type 2 diabetes. To date, about 80 loci have been linked to type 2 diabetes risk, but their individual effects are modest.[50] Although the biological functions are to a large extent unknown, most of these genetic variants seem to be related to β-cell function rather than insulin action.[48]

Besides genetic susceptibility, important non-modifiable risk factors include increasing age, history of gestational diabetes, and polycystic ovarian syndrome.[51]

2.1.6.2 Modifiable factors

Among the modifiable factors related to type 2 diabetes risk, obesity is singled out as the most important.[51] Excess body fat, in particular centrally accumulated, is strongly associated with insulin resistance, and most people diagnosed with type 2 diabetes are overweight or obese.[52] Physical inactivity is also a key risk factor, even independently of its effects on body composition.[51] Exercise acutely activates non-insulin dependent glucose uptake in skeletal muscle, and regular physical activity leads to adaptations that improve long-term insulin sensitivity.[53] Other lifestyle-related risk factors of importance include unhealthy dietary habits, smoking, and alcohol consumption.[51] Factors such as psychological distress and work stress are also associated with higher risk.[54, 55]

As previously noted, prediabetes and the metabolic syndrome are major risk factors for type 2 diabetes development. These conditions are themselves largely influenced by the same modifiable risk factors.[51] Several trials have demonstrated that progression to type 2 diabetes among people with prediabetes can be efficiently prevented with lifestyle interventions focusing on diet and physical activity.[56, 57]

Specific dietary factors

Over the past decades, evidence on associations between specific foods and dietary factors in relation to risk of type 2 diabetes has accumulated, in particular from prospective cohort studies. With the rapid accumulation of evidence, meta-analysis has become an important tool to summarize results from different studies. Prospective studies on type 2 diabetes risk have been summarized in recent meta-analyses for most major food groups (**Figure 2.2**).

Red meat is among the foods that have been most consistently associated with higher risk of type 2 diabetes. In a meta-analysis from 2011, including nine cohort studies on unprocessed red meat and eight on processed red meat consumption, direct associations were observed for both types.[58] Several studies have been published since, of which some have been in line with this conclusion,[59-61] whereas others have observed direct associations only for processed red meat.[19, 62, 63] A possible explanation behind the association relates to the heme iron content of red meat. Iron is a strong prooxidant, and both heme iron intake and high body iron stores have been associated with higher risk of type 2 diabetes.[64, 65] Higher sodium content or preservatives such as nitrites and nitrates have been proposed to explain the stronger association with processed meat.[66, 67]

Whole grain consumption was strongly associated with lower type 2 diabetes risk in a meta-analysis from 2013.[68] In the same study, no association was observed for total **refined grain** consumption, whereas specifically white rice was associated with higher risk. A separate meta-analysis on white rice consumption concluded that there was a stronger direct association in Asian populations where white rice is a major calorie source.[69] Benefits of whole grain foods relates to the high fiber content and low glycemic index, which contributes to a more controlled elevation of postprandial blood glucose. On the contrary, refined grains have a high glycemic index, and a generally reduced nutrient content.

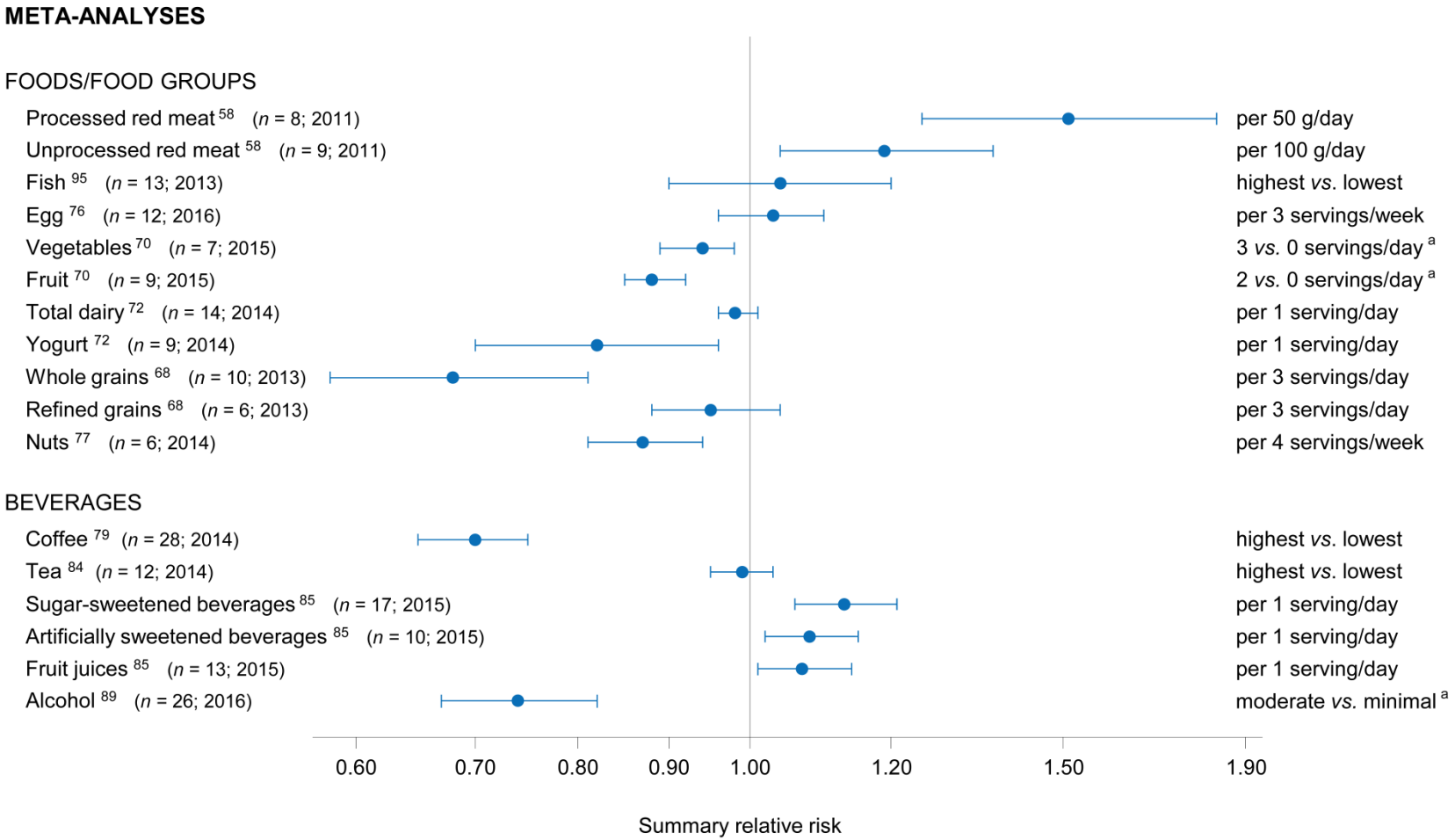


Figure 2.2 Overview of summary relative risks (with horizontal lines representing 95% confidence intervals) from the most recently published meta-analyses of prospective studies on foods/food groups and beverages in relation to type 2 diabetes risk (*n*: number of studies included in meta-analysis; year of publication)

[a] nonlinear association; relative risk shown for the consumption level most strongly associated with type 2 diabetes risk

A meta-analysis of prospective cohort studies of **fruit and vegetable** consumption from 2015 reported nonlinear associations. Compared with consumption less than daily, the lowest type 2 diabetes risks were observed for three servings per day of vegetables and two servings per day of fruit, whereas no statistically significant associations were observed for higher consumption levels.[70] The benefits of fruit and vegetable consumption may further differ by subtypes. Higher consumption of berries, green leafy vegetables, yellow vegetables, and cruciferous vegetables was associated with lower type 2 diabetes risk in another recent meta-analysis.[71] Fruit and vegetables are rich in dietary fiber and a wide range of compounds with antioxidative properties which could explain the inverse associations.[71]

Among **dairy foods**, only yogurt was associated with lower type 2 diabetes risk in a meta-analysis from 2014.[72] This could possibly be explained by the content of probiotic bacteria, which have been shown to have beneficial effects on lipid profile and antioxidant status.[73, 74] The inverse association with yogurt was supported in the only subsequently published cohort study.[75]

The role of **egg** consumption has been debated, with several studies in US populations suggesting a direct association with type 2 diabetes. As concluded in recently published meta-analysis, this association has not been supported in studies conducted in other regions, and the discrepancies could potentially be explained by differences in egg consumption habits and associated overall dietary patterns.[76]

Nuts have been suggested to have beneficial effects because of their unsaturated fat content, and higher consumption was associated with lower type 2 diabetes risk in a meta-analysis from 2014.[77] In addition to five cohort studies, this meta-analysis also included the PREDIMED (Prevención con Dieta Mediterránea) trial, in which supplementation of nuts significantly reduced incident diabetes in the context of a Mediterranean diet.[78]

Among beverages, **coffee** consumption has been extensively studied in relation to type 2 diabetes, and has been consistently associated with a lower risk.[79-82] The inverse association has been observed for both caffeinated and decaffeinated coffee, indicating that other bioactive compounds may be of importance. Although a wide range of potential mediators have been investigated, the association has only been partly explained.[83]

The association with **tea** consumption is less established, and results from prospective cohort studies have been largely inconsistent. A meta-analysis from 2014 found no significant relationship with type 2 diabetes overall, but an inverse association in studies evaluating consumption as high as three cups or more per day.[84]

Sweetened beverage consumption has been associated with a higher risk of type 2 diabetes. In a meta-analysis published in 2015, a direct association was reported for sugar-sweetened beverage consumption, independently of adiposity.[85] Direct associations were observed also for artificially sweetened beverages as well as for fruit juices, although these were less

consistently supported in sensitivity analyses. The only subsequent cohort study on these associations was consistent with the conclusion on sugar-sweetened beverages, whereas no association was observed for fruit juices, and a positive association with artificially sweetened beverages was attenuated after adjustment for adiposity.[86] The proposed mechanisms of a positive association mainly relate to the sugar content, but there may also be effects of food colorings, phosphoric acid, or other constituents.[87]

Moderate **alcohol** consumption has been suggested to confer benefits with regard to type 2 diabetes development. The proposed mechanisms relate to increases in insulin sensitivity, increases in HDL cholesterol levels, or alterations of inflammatory proteins.[88] The most recent meta-analysis on this topic was published earlier in 2016, and included 26 prospective observational studies. Light and moderate alcohol consumption was associated with lower risk of type 2 diabetes, whereas no association was observed for heavy consumption. The lower risk was observed at less than 20 grams per day for women and less than 40 grams per day for men.[89]

The results on **fish** consumption in relation to type 2 diabetes risk have been largely inconsistent between published prospective studies,[90-96] and are discussed more in detail in section 2.2 and 5.1.

Dietary scores and patterns

Adherence to diet quality scores such as the Dietary Approaches to Stop Hypertension (DASH) diet, the Alternate Healthy Eating Index (AHEI), the Mediterranean diet, and the Healthy Nordic Food Index, has also been associated with lower risk of type 2 diabetes.[97-99] In these scores a number of pre-specified 'healthy' and 'unhealthy' dietary factors are combined to obtain an overall estimate of diet quality. Another approach to assess overall diet is a posteriori defined dietary patterns, for which statistical methods are used to cluster dietary factors based on the data at hand. A recent meta-analysis of prospective studies using such approaches showed that adherence to 'healthy' dietary patterns (generally defined by higher consumption of vegetables, fruits, whole grains, low-fat dairy, fish, poultry, and legumes) was associated with lower type 2 diabetes risk, while adherence to 'unhealthy' patterns (generally defined by higher consumption of red meat, fried and processed foods, refined grains, sweets and desserts) was associated with higher type 2 diabetes risk.[100]

2.2 FISH CONSUMPTION

Low rates of cardiometabolic diseases in Greenlandic Inuit populations with high fatty fish consumption sparked an interest in the health effects of fish already in the 1970's.[101] Today, many national agencies and organizations around the world, including the Swedish National Food Agency, recommend at least two servings of fish per week, with an emphasis on fatty fish rich in omega-3.[102, 103] These recommendations primarily relate to coronary heart disease (CHD) risk reduction. In addition to long-chain omega-3 fatty acids, fish contains several other nutrients that may be hard to obtain in sufficient amounts from other food sources. Fish may also serve as a good source of high quality protein, and as an alternative to excessive red meat consumption. It is however important to note that fish is the major dietary source of exposure to some environmental contaminants whose health effects remain to be fully elucidated.[104, 105] Although not further addressed in this thesis, it should also be noted that issues of sustainability add to the complexity of the topic of fish consumption.[106]

2.2.1 Nutritional value

2.2.1.1 Long-chain omega-3 fatty acids

Fatty fish is the major food source of long-chain omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Smaller amounts of the EPA metabolite docosapentaenoic acid (DPA) are also present in fish.[107] These fatty acids have unique properties and distinct biological functions due to their structure with a long carbon chain and the first double bond at three carbon atoms from the methyl terminal.[108] The shorter-chain omega-3 fatty acid alpha-linolenic acid (ALA), which is present in fatty plant foods such as seeds and nuts, can only to a limited extent be converted to EPA and DHA. Thus, direct dietary intake is the major determinant of circulating long-chain omega-3 fatty acid levels.[107]

The long-chain omega-3 fatty acids exert their biological functions in various ways, including modulation of eicosanoid pathways, modulation of signaling pathways, incorporation into cell membranes, and effects on gene expression.[108] They may influence cardiometabolic health by reducing plasma triglycerides, inflammation, heart rate, blood pressure, and platelet aggregation, preventing arrhythmias and improving endothelial function.[107]

The effects of long-chain omega-3 fatty acid supplementation on major cardiovascular disease endpoints have however been less evident,[109] suggesting that potential benefits of fish consumption indicated in observational studies may be attributed to a wider range of nutrients. The overall evidence from supplementation trials in specific populations of patients with impaired glucose metabolism do not support an effect on major cardiovascular events or mortality in this group.[110] With regard to insulin sensitivity and glucose control, most trials have shown no effect.[110-112] For type 2 diabetes incidence, no randomized controlled trials on the effect of long-chain omega-3 fatty acid supplementation have been carried out to date.

Circulating levels of long-chain omega-3 fatty acids were not associated with risk of type 2 diabetes in the prospective studies included in a 2012 meta-analysis.[92] Among subsequent studies examining circulating biomarkers, inverse associations were observed in some,[18, 113, 114] but not all.[115-117]

2.2.1.2 Vitamin D

Vitamin D is synthesized in the skin upon exposure to ultraviolet-B radiation. Other sources are however required to maintain adequate levels during winter at high latitudes. Few food sources contain significant amounts of vitamin D, and fatty fish is one of the most important contributors.[118] Vitamin D has long been known to play an important role for bone health, with low levels associated with osteomalacia, lower bone mineral density, and higher risk of fractures. More recently, accumulating evidence has associated vitamin D with several other diseases.[119]

Although inadequate vitamin D status has been observationally linked to higher type 2 diabetes incidence,[120] the causality of this association has been questioned.[121] As summarized in a 2014 meta-analysis, most randomized controlled trials of vitamin D supplementation have not provided support for an effect on glucose homeostasis or diabetes prevention.[122] Observational evidence also associates low vitamin D status with higher risk of all-cause and cardiovascular mortality, as well as with incident CHD and stroke. Limited trial evidence on cardiovascular disease endpoints available to date is however inconclusive.[118]

2.2.1.3 Selenium

The selenium content of plant foods vary depending on the content of the soil. In areas with low soil content, such as in Sweden, fish is an important contributor to selenium intake.[123] Selenium is an essential trace nutrient and a component of several selenoproteins of importance for the body's antioxidative defense. Adequate selenium status may augment the defense system, but the beneficial dose range is relatively narrow. Supplement trials evaluating effects on glucose homeostasis and type 2 diabetes incidence have shown discrepant results that seem to depend on differences in baseline selenium status.[124] For cardiovascular disease outcomes, a 2016 meta-analysis concluded that the overall observational evidence indicates an inverse association with selenium status, whereas randomized controlled trials do not support an effect of supplementation.[125]

2.2.1.4 Protein

Fish stands out among other animal foods by containing high amounts of high quality protein for low amounts of saturated fat. In addition, some of the specific amino acids found in high amounts in fish, such as arginine, glutamine, and taurine, have been shown to have beneficial effects on glucose tolerance, insulin sensitivity, and cardiovascular disease risk.[2, 126]

2.2.1.5 Other nutrients

Fish is further a good source of other nutrients with potentially favorable effects on cardiometabolic health, including iodine,[127] magnesium,[128-130] potassium,[131, 132] calcium,[130, 133, 134] and B vitamins.[135-137]

2.2.2 Environmental contaminants

2.2.2.1 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are highly persistent fat-soluble compounds that were industrially produced during the 20th century. Although production was banned in most parts of the world in the late 1970's, PCBs remain widespread in the environment.[138] As they accumulate and magnify in the food chain, diet constitutes the major part of PCB exposure in the general population. Most of the dietary exposure originates from animal food sources, and the highest amounts are found in fish.[139, 140] In Sweden, high levels have been observed in fatty fish from the Baltic Sea and contaminated lakes,[140] and sensitive groups (children, adolescents, women of childbearing age, as well as pregnant and nursing women) are advised to limit their consumption of fatty fish from these waters.[103]

Exposure to persistent organic pollutants such as PCB has been shown to induce insulin resistance in animal studies.[141] In humans, circulating levels have been associated with higher risk of type 2 diabetes as well as cardiovascular diseases and associated risk factors.[142-144] Dietary PCB exposure has been associated with higher risk of MI and stroke in the cohorts studied in this thesis.[145-147]

2.2.2.2 Methylmercury

Mercury is a heavy metal, emitted into the atmosphere mainly from processes such as combustion of coal, waste incineration, smelting, and mining activities. To a smaller degree, it is also released from natural sources such as volcanic activity. Inorganic mercury is converted to organic methylmercury (MeHg) by microorganisms in lakes, rivers, and oceans. MeHg bioaccumulates in the food chain and can be found in high amounts in large predatory fish in some waters.[148] High MeHg exposure, especially during fetal development, can damage the central nervous system. For this reason, the Swedish National Food Agency advices women who are pregnant, nursing, or planning a pregnancy to limit consumption of predatory fish species such as perch, pike, pikeperch, and tuna.[103]

Due to its oxidative properties, MeHg has been proposed to have adverse effects on cardiometabolic health. In experimental studies, MeHg exposure has been shown to induce β-cell dysfunction,[149] and to have a variety of effects related to cardiovascular risk.[150] Epidemiological findings on MeHg exposure are however inconsistent in relation to both type 2 diabetes risk[18, 151, 152] and cardiovascular disease outcomes.[153-156]

2.2.3 Prospective studies on fish consumption in relation to type 2 diabetes incidence

The body of evidence from prospective cohort studies on the relationship between fish consumption and risk of type 2 diabetes has emerged over the past decade. The observed associations have ranged from inverse[14, 15] to direct,[8, 10, 13] with most studies reporting no statistically significant association with total fish consumption.[6, 7, 9, 11, 12, 16-19] Results from our meta-analysis of these findings are presented in section 5.1. Various aspects of fish consumption that have not been assessed in many of these studies, such as type of fish, preparation methods, and degree of contamination, may be of importance in explaining the differences and understanding the potential role of fish consumption in type 2 diabetes development.

2.2.4 Prospective studies on fish consumption in relation to cardiovascular disease and mortality among people with diabetes

A relatively large body of observational evidence supports an inverse association between fish consumption and risk of major cardiovascular events such as CHD[3, 4] and stroke,[5] as well as overall mortality[157] in the general population. Studies specifically in populations with prevalent diabetes are however more scarce.

A strong inverse association with incident CHD events was observed among women with baseline type 2 diabetes in the Nurses' Health Study.[20] In a smaller study of 117 cases, fish consumption was not associated with CHD risk.[21] In a cohort of coronary artery disease patients, there was no clear associations between fish consumption and incidence of MI, either among those with or without prevalent diabetes.[22] Dietary long-chain omega-3 fatty acid intake was however associated with lower risk of MI only among those with diabetes.[22] No studies have evaluated fish consumption in relation to stroke incidence among participants with prevalent diabetes.

Three studies have assessed fish consumption in relation to total mortality in populations with diabetes. In the Nurses' Health Study, a strong inverse association was observed, similar to the one reported for incident CHD events.[20] A modest statistically nonsignificant inverse association was observed in another US cohort.[158] A smaller study including only 80 deaths reported no association.[159]

3 AIMS

The overall aim of this thesis was to evaluate fish consumption in relation to incident type 2 diabetes as well as subsequent cardiovascular complications and mortality.

The specific aims were:

- to summarize results from published prospective studies on the associations of fish consumption and dietary intake of long-chain omega-3 fatty acids with risk of type 2 diabetes (**paper I**)

- to evaluate the validity of food frequency questionnaire (FFQ)-based estimates of long-chain omega-3 fatty acid intake and fish consumption (**paper II**)

- to examine the associations of total fish consumption, fried fish, and specific fish items with risk of type 2 diabetes, taking exposure to environmental contaminants present in fish into consideration (**paper III**)

- to assess consumption of total and specific types of fish in relation to risk of myocardial infarction (MI), stroke, and mortality among people with diagnosed type 2 diabetes (**paper IV**)

4 SUBJECTS AND METHODS

Paper I in this thesis is a systematic review and meta-analysis of previously published evidence from prospective studies. **Papers II – IV** are based on data from two population-based cohorts: the Swedish Mammography Cohort (SMC) and the Cohort of Swedish Men (COSM).

4.1 META-ANALYSIS

4.1.1 Search strategy

The PubMed and EMBASE databases were searched for relevant articles published up to 15 December 2011, using the search term "diabetes" combined with "fish", "seafood", "long-chain omega-3", "long-chain n-3", "docosahexaenoic acid", "eicosapentaenoic acid", or "fatty acids" and "cohort", "prospective", "follow-up", or "longitudinal". No language or other restrictions were imposed. Reference lists of retrieved articles were searched for additional relevant studies. For this thesis, an updated search was performed up to 29 February 2016.

4.1.2 Study selection

The following inclusion criteria were applied: a) prospective study design; b) exposure of interest: fish consumption or dietary intake of long-chain omega-3 fatty acids; c) outcome of interest: incidence of type 2 diabetes; and d) relative risk (RR) estimates with 95% confidence intervals (CIs) reported. Odds ratios, risk ratios, and hazard ratios were considered estimates of the RR. If data from the same cohort were duplicated in more than one article, the one based on the largest number of cases was included.

4.1.3 Data extraction

From each study, we extracted the first author's last name, publication year, study country, study period, study size, sex and age of participants, categorization of exposure, methods for exposure assessment and type 2 diabetes ascertainment, variables adjusted for in the analysis, and RR estimates with corresponding 95% CIs for each consumption category. If results from several multivariable models were reported, we extracted the risk estimates representing the highest degree of control for potential confounders. To obtain all necessary data, we contacted authors of studies that did not report information on the distribution of cases and

non-cases/person-time, or that presented RRs for only two exposure categories. Additional data were supplied by the authors of all five studies for which requests were made.[6, 9, 11, 15, 160]

4.1.4 Quality assessment

Study quality was assessed using the Newcastle-Ottawa Quality Assessment Scale for cohort studies.[161] Each study was evaluated based on eight questions grouped into *selection* (representativeness of the exposed, selection of non-exposed, exposure ascertainment, exclusion of prevalent cases; 0–4 points), *comparability* (control for important covariates; 0–2 points), and *outcome* (assessment of outcome, length of follow-up, completeness of follow-up; 0–3 points).

4.1.5 Statistical analysis

We combined the results from published studies using a two-stage dose–response random effects meta-analysis. In the first stage, study-specific linear dose–response curves were estimated using a method that takes into account that the RRs for different exposure levels are not independent.[162] For each category of exposure, the median or midpoint consumption was assigned as the dose for the corresponding RR estimate. Open ended consumption categories in the lower end were assumed to range from zero, and in the higher end to be of the same magnitude as the preceding category. To rescale fish consumption reported in grams into servings, we used 100 grams as an approximate average serving size. In the second stage of the meta-analysis, the study-specific trend estimates were combined.[163] Potential departure from linearity in the relationship was assessed using restricted cubic splines with three knots at 10, 50, and 90% of the distribution. Statistical heterogeneity was evaluated with the Cochran Q-test and the I^2 statistic, which quantifies the proportion of total variation that is explained by between-study variation.[164] To explore sources of heterogeneity, we performed stratified analyses by sex, geographical region, methods for exposure and outcome assessment, and quality score. Sensitivity analyses included omission of one study at the time to evaluate if single studies markedly influenced the results, omission of studies with nested case-control design, and altering the fish serving size to 80 or 140 g for rescaling of exposure measures to servings. To assess publication bias, we used the Egger regression asymmetry test.[165]

4.2 COHORT STUDIES

4.2.1 Study population

Papers II – IV in this thesis are based on data from two population-based cohort studies, the SMC and the COSM, consisting of women and men from three counties in central Sweden (**Figure 4.1**). The studies have been approved by the Regional Ethical Review Board in Stockholm, Sweden, and all participants have given written informed consent. The cohorts have been shown to be representative of Swedish women and men of the same age, in terms of educational level, prevalence of overweight, and age distribution.[166] **Figure 4.2** provides an overview of the data collection and exclusions made to obtain the final study populations.

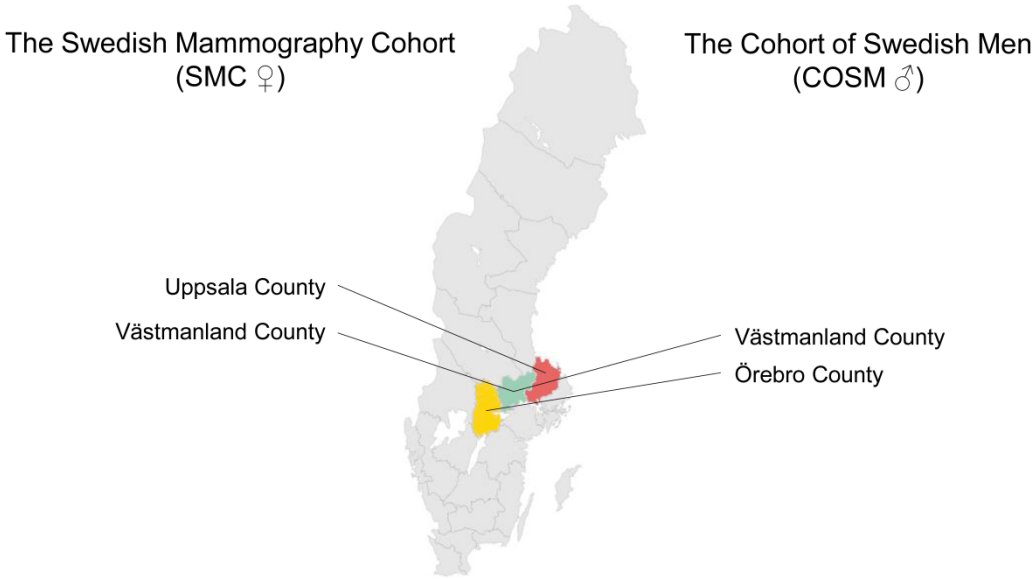


Figure 4.1 Study area for the Swedish Mammography Cohort and the Cohort of Swedish Men

4.2.1.1 The Swedish Mammography Cohort

The SMC was established in 1987–1990. All women born between 1914 and 1948, resident in the counties of Uppsala and Västmanland, received an invitation to participate along with a self-administered questionnaire concerning diet and other lifestyle factors (response rate 74%). In 1997, a second questionnaire was sent to all participants still alive and living in the study area (response rate 70%). The 1997 questionnaire version was expanded to include more food items as well as information on prevalent diabetes and important lifestyle factors such as cigarette smoking, physical activity, and dietary supplement use. In 2008, a new questionnaire on health, including a question on diabetes status, was distributed (response rate 63%).

Between 2003 and 2009, cohort participants living in Uppsala were successively recruited to the SMC-clinical subcohort (SMC-C). Upon return of an additional food frequency questionnaire (FFQ), the women were invited to Samariterhemmet in Uppsala for a clinical

visit where fasting samples of blood, urine, and adipose tissue were collected. Weight and height were measured by a nurse, and measurements of bone mineral density and body composition were performed. Women who participated in phase 1 of the SMC-C (between 2003 and 2004; n = 465) completed the same FFQ version as distributed to the whole cohort in 1997 and were eligible for inclusion in **paper II.** Adipose tissue samples were collected from 366 of these women (79%). Among these, 240 samples were randomly selected for analysis of fatty acid composition. One woman was excluded because she had returned a blank FFQ, resulting in a final study population of 239 women.

In **paper IV**, we used data from the women who returned the 1997 FFQ. After exclusion of those who filled in an incorrect personal identity number, those who died, had a diagnosis of cancer (not including non-melanoma skin cancer), or cardiovascular disease (myocardial infarction [MI], angina, or stroke) before 1 January 1998, those with implausible total energy intake (±3 SD from the log-transformed mean), and those with missing FFQ data on all items in the fish consumption section or on frying frequency, 32,742 women remained. Among these, 912 women had a diagnosis of type 2 diabetes registered in the Swedish National Diabetes Register or the Swedish National Patient Register before 1 January 1998 and were included in the final study population.

4.2.1.2 The Cohort of Swedish Men

The COSM was established in the autumn of 1997, when all men born between 1918 and 1952 and resident in the counties of Västmanland and Örebro were invited to participate. They received a self-administrated questionnaire, which with the exception of some sex-specific questions was the same as the one distributed to SMC in 1997 (response rate 49%). An extended questionnaire on health, including a question on diabetes status, was sent out in 2008 (response rate 78%).

Among the men who returned the 1997 FFQ, 38,066 were left after exclusion of those who filled in an incorrect personal identity number, those who returned a blank questionnaire, those who died, had a diagnosis of cancer (not including non-melanoma skin cancer), or cardiovascular disease (MI, angina, or stroke) before 1 January 1998, those with implausible total energy intake (±3 SD from the log-transformed mean), and those with missing FFQ data on all items in the fish consumption section or on frying frequency.

In **paper III**, men without any diabetes diagnosis before baseline (based on register data and self-reports) were considered. We further excluded those who reported a diabetes diagnosis in the 2008 questionnaire that was not confirmed by register data, and those who were registered with diabetes other than type 2 during follow-up, resulting in a final study population of 35,583 men.

In **paper IV**, the 1,313 COSM men with a diagnosis of type 2 diabetes registered in the Swedish National Diabetes Register or the Swedish National Patient Register before 1 January 1998 were included, and pooled with the 912 SMC women.

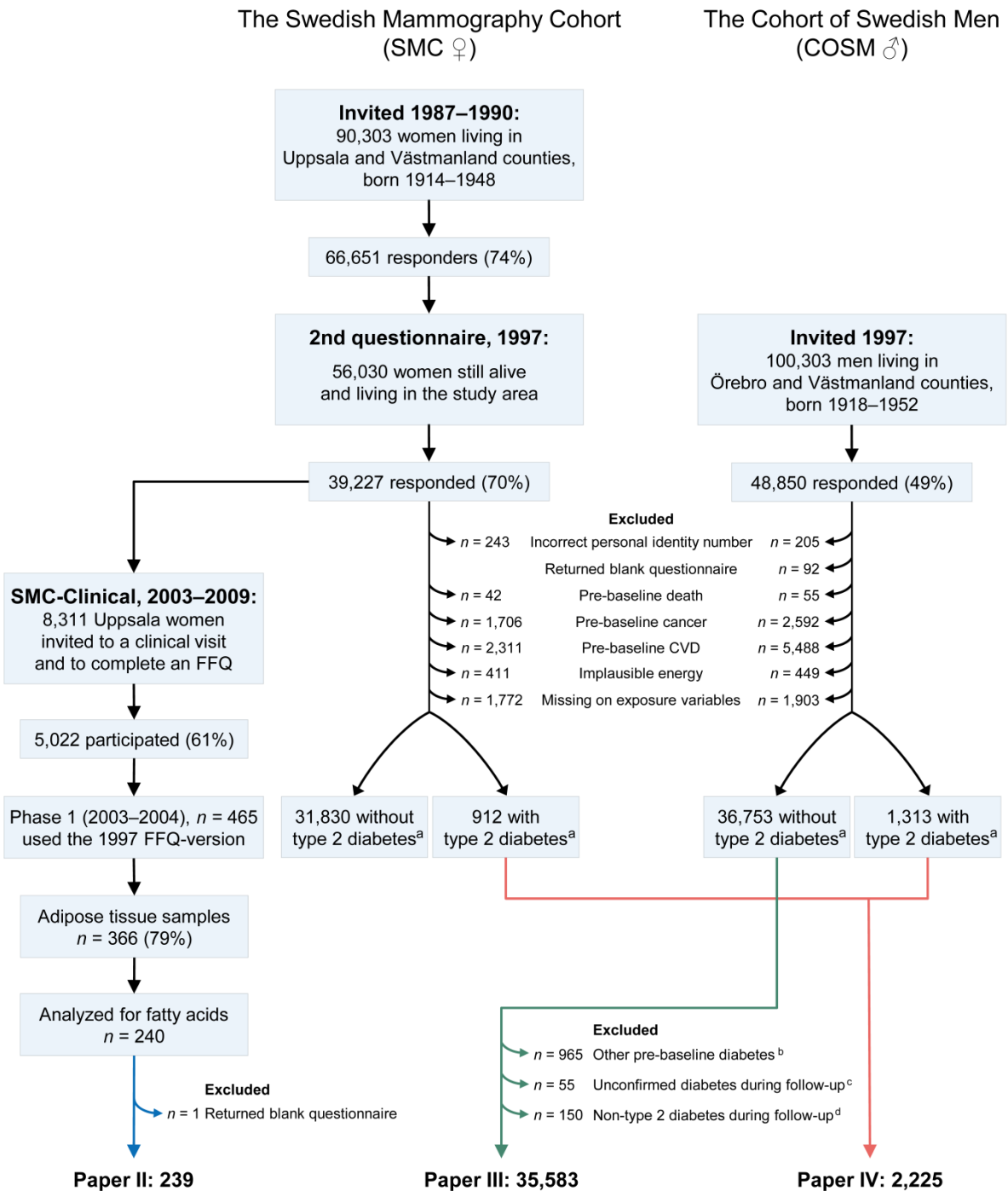


Figure 4.2 Data collection and exclusions for **papers II – IV**. *CVD*, cardiovascular disease; *FFQ*, food frequency questionnaire

[a] registered diagnosis of type 2 diabetes in the Swedish National Diabetes Register or the Swedish National Patient Register before 1 January 1998

[b] includes registered diagnoses of diabetes other than type 2 in the Swedish National Diabetes Register or the Swedish National Patient Register before 1 January 1998, and self-reported prevalent diabetes in the 1997 questionnaire

[c] self-reported prevalent diabetes in the 2008 questionnaire but no diagnosis record in the Swedish National Diabetes Register or the Swedish National Patient Register

[d] registered diagnosis of diabetes other than type 2 in the Swedish National Diabetes Register or the Swedish National Patient Register between 1 January 1998 and 31 December 2012

4.2.2 Exposure and covariate assessment

4.2.2.1 Questionnaire-based estimates of exposures and covariates

The major part of the exposure data was collected via self-administered questionnaires, including extensive sections on diet, as well as questions on non-dietary lifestyle factors and health status. Dietary intake was assessed with semi-quantitative FFQs. This thesis is based mainly on the FFQ data from 1997 (FFQ-97), which is the baseline for the disease association studies (**paper III** and **paper IV**). In **paper II**, we additionally used data from two other FFQ assessments: the initial SMC recruitment in 1987–1990 (FFQ-87) and phase 1 of the SMC-C in 2003–2004 (FFQ-03).

The FFQ-87 was a less extensive version including 67 food items. Participants were asked to report their average consumption of these foods over the past six months, choosing from eight predefined frequency categories ranging from never/seldom to four times per day or more. FFQ-97 and FFQ-03 were identical questionnaire versions, including 96 food items. Participants were queried about average consumption of these foods over the previous year, choosing from eight predefined frequency categories that ranged from less than one time per month to three times per day or more.

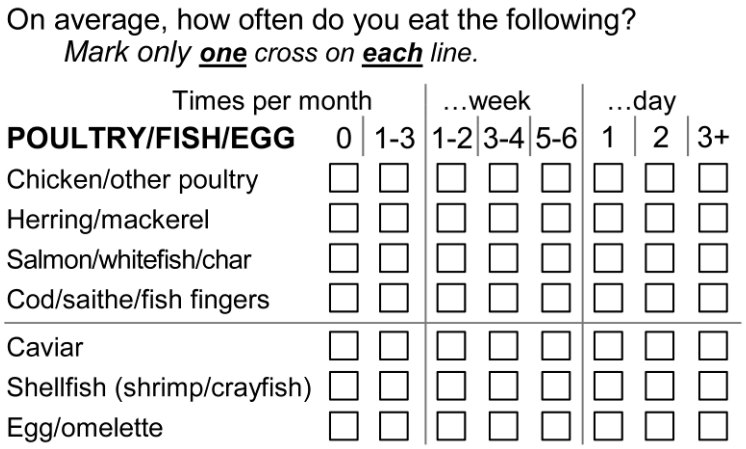
For each food item, consumption expressed in servings per day was derived from the midpoint of the chosen frequency range. Consumption in grams per day was calculated based on age-specific portion sizes (≤52, 53–61, 62–69, ≥70 years for men; ≤52, 53–65, ≥66 years for women). The portion sizes were derived from weighted food records kept by random samples of 152 men and 213 women from the study area. For calculation of total energy and nutrient intakes, we used food composition values obtained from the Swedish National Food Agency database, which is based on analyses of representative foods on the Swedish market and takes into account cooking losses for prepared foods and dishes.[167] For each FFQ item, nutrient calculations were based on an aggregate of a larger number of specific food types according to the distribution of consumption in the population. Nutrient intakes were further adjusted to the mean energy intake in the cohorts using the residual method.[168]

Fish consumption

FFQ-87 included two items on finfish consumption (salmon/mackerel/herring, other fish) and one item on shellfish (shrimp/mussels/crab). In FFQ-97/FFQ-03, three finfish items (herring/mackerel, salmon/whitefish/char, cod/saithe/fish fingers) and one item on shellfish (shrimp/crayfish) were included (**Figure 4.3**).

In **paper III** and **paper IV**, non-response on fish items was assumed to imply never/seldom for participants who reported consumption of other items in the poultry/fish/egg section. Non-responders of the entire section were left with missing values on fish consumption and excluded from the study populations. These assumptions were based on a study in a Swedish

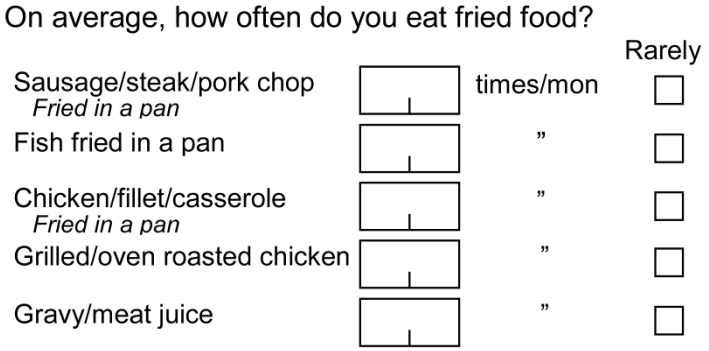
population showing that 82% of missing reports on fish consumption corresponded to actual non-consumption.[169] For the correlation analyses of fish consumption in **paper II**, all non-response was treated as non-consumption.


On average, how often do you eat the following?
*Mark only **one** cross on **each** line.*

	Times per month		…week			…day		
POULTRY/FISH/EGG	0	1-3	1-2	3-4	5-6	1	2	3+
Chicken/other poultry	☐	☐	☐	☐	☐	☐	☐	☐
Herring/mackerel	☐	☐	☐	☐	☐	☐	☐	☐
Salmon/whitefish/char	☐	☐	☐	☐	☐	☐	☐	☐
Cod/saithe/fish fingers	☐	☐	☐	☐	☐	☐	☐	☐
Caviar	☐	☐	☐	☐	☐	☐	☐	☐
Shellfish (shrimp/crayfish)	☐	☐	☐	☐	☐	☐	☐	☐
Egg/omelette	☐	☐	☐	☐	☐	☐	☐	☐

Figure 4.3 Food frequency questionnaire section on fish consumption (FFQ-97/FFQ-03)

Total fish consumption was calculated as the sum of herring/mackerel, salmon/ whitefish/char, and cod/saithe/fish fingers. In **paper II,** as well as for the updated meta-analysis presented in section 5.1.5, the sum of herring/mackerel and salmon/whitefish/char was used as an estimate of fatty fish consumption. In a separate FFQ section specifically on fried foods, participants were ask to report average monthly frequency of consuming fish fried in a pan (**Figure 4.4**).


On average, how often do you eat fried food?

			Rarely
Sausage/steak/pork chop *Fried in a pan*	☐	times/mon	☐
Fish fried in a pan	☐	”	☐
Chicken/fillet/casserole *Fried in a pan*	☐	”	☐
Grilled/oven roasted chicken	☐	”	☐
Gravy/meat juice	☐	”	☐

Figure 4.4 Food frequency questionnaire section on fried foods (FFQ-97/FFQ-03)

Validity of the FFQs has been evaluated in random samples of women and men from the study area. Among 248 men who completed fourteen repeated 24-h recall interviews during a one-year period, the correlations with FFQ-based estimates ranged from 0.44 to 0.81 for macronutrients and from 0.38 to 0.81 for micronutrients. For eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the correlation coefficients were 0.64 and 0.60, respectively.[170] Food items have been validated among 129 women, with correlations between the FFQ-based estimates and those from four one-week weighted diet records of 0.5 for fatty fish, 0.4 for lean fish, and 0.6 for shellfish (A. Wolk, unpublished data). Validity of FFQ-based estimates of fish consumption and dietary long-chain omega-3 fatty acid intake in comparison with adipose tissue content was assessed in **paper II**, and the results are presented in section 5.2.

Other dietary factors

To account for overall diet quality in the analyses of **paper III** and **paper IV**, we used a quality score instead of single food groups. We chose the DASH (Dietary Approaches to Stop Hypertension) diet component score proposed by Fung et al,[171] as it has been associated with lower risk of type 2 diabetes and does not include fish consumption. The score was calculated based on reported consumption of eight dietary components. Higher points were awarded for high consumption of fruits, vegetables, nuts and legumes, low-fat dairy, and whole grain foods, and for low consumption of sodium, sweetened beverages, and red and processed meat.

Dietary contaminant exposure

For calculations of dietary exposure to polychlorinated biphenyls (PCBs) and methylmercury (MeHg), we used recipe-based databases previously created for the FFQ-97. Concentrations of PCBs and MeHg in foods were obtained from the Swedish National Food Agency and the Swedish Environmental Protection Agency's monitoring programs. The PCB database was based on concentration of congener 153 in over 1,200 food samples. PCB153 is the most abundant PCB congener in Swedish foods and an excellent indicator for total PCB. Time trends of PCB concentrations in food were taken into account to reflect concentration at baseline.[104] The MeHg database was based on concentration data from 1,353 fish samples analyzed between 1975 and 2007, without any obvious time trend.[172] Based on the FFQ responses, total dietary PCB and MeHg exposures were then calculated following the procedures for nutrient calculations described above (i.e. using age-specific portion sizes, aggregated codes, and energy-adjustment).

The FFQ-based estimates of dietary PCB exposure have been validated in a subsample of 201 SMC-C women. The correlation between the dietary exposure estimates and the sum of six PCB congeners measured in serum was 0.48.[104] In another study of a Swedish population, a correlation of 0.75 was observed between FFQ-estimated fish consumption and hair mercury.[105]

Other health and lifestyle factors

Information on other health and lifestyle factors, including body weight, height, education, alcohol consumption, smoking habits, physical activity, history of high cholesterol, history of hypertension, family history of MI, use of aspirin, and use of fish oil supplements was also self-reported in the 1997 questionnaire.

Body mass index (BMI; kg/m^2) was calculated from reported values on weight and height. Physical activity was assessed with questions on active time spent doing home/household work, walking/bicycling, and exercising; on inactive time spent watching TV/reading and sleeping; and on level of occupational physical activity. In **paper III**, we used a score of total physical activity calculated by multiplying the reported amounts of these activities by their typical energy expenditure requirements expressed in metabolic equivalents (METs).[173] In **paper IV**, we instead used the walking/bicycling variable as a proxy for physical activity.

4.2.2.2 Clinical data and biomarker analysis

In **paper II**, we used data collected within the SMC-C. Subcutaneous adipose tissue samples of 5–25 mg were taken from the buttock with a fine needle attached to a vacuum tube and then stored at −80 °C until analyzed.[174] Total fat mass and total lean mass were measured using dual-energy X-ray absorptiometry (DXA; Lunar Prodigy, Lunar Corp., Madison, WI, USA). The precision error of the measurements was 1.5% for total fat mass and 1.0% for total lean mass. These measurements were used to calculate body fat percentage.

The adipose tissue samples were analyzed for fatty acid composition in 2011. After extraction and transmethylation, the fatty acids were separated by gas–liquid chromatography on a 30 m glass capillary column coated with Thermo TR-FAME (Thermo Electron Corporation, Waltham, MA, USA), with helium as a carrier gas. An Agilent Technologies system (Santa Clara, CA, USA) with temperature programmed to 150–260 °C was used. Individual fatty acids were identified by comparing retention times with fatty acid methyl ester standard Nu Check Prep (Elysian, MN, USA). The amount of each fatty acid was expressed as weight percentage of the sum of all fatty acids.

4.2.3 Ascertainment of disease

Using the Swedish personal identity number, the cohorts were linked to several national registers to identify participants with prevalent disease for exclusion purposes, as well as incident cases of the outcomes of interest in **paper III** and **paper IV**.

4.2.3.1 The National Diabetes Register

The Swedish National Diabetes Register was initiated in 1996 as a tool for quality assurance in diabetes care. It contains clinical information from patient visits in specialized as well as primary care, reported by trained physicians and nurses. Reporting is not mandatory, but highly encouraged. In 2013, 100% of specialist clinics and 95% of primary health care centers participated.[175]

Since initiation in 1996, the coverage has gradually improved.[24, 176] Based on an expected diabetes prevalence of 4% of adults, the national coverage is now estimated to about 97% with some variation across the country. Västmanland and Örebro counties reach complete coverage based on this estimation, while about 62% are covered in Uppsala County. In comparison with the Swedish Prescribed Drug Register, it has been shown that about 90% of people aged 50–80 years on diabetes medication were covered (Västmanland: 95%; Örebro: 92%; Uppsala: 77%).[24] Of note, the Diabetes Register includes a retrospective recording of debut year, allowing us to backdate the diabetes diagnosis for cohort participants that were first registered in the later years.

The Diabetes Register defines type of diabetes in two ways. According to the *epidemiological* classification, patients diagnosed before the age of 30 years and with insulin treatment only are classified as type 1. Patients diagnosed at the age of 40 years or older, as well as patients at any age treated only with diet and/or oral agents are classified as type 2. Diabetes type is also reported according to the *clinical* assessment as type 1, type 2, or secondary diabetes.

4.2.3.2 The National Patient Register

The Swedish National Patient Register at the National Board of Health and Welfare was initiated in 1964 to collect data on health care use through physician reports. Since 1987, it has been nationwide with mandatory reporting for all in-patient care. The study counties, Uppsala, Örebro, and Västmanland, have been fully covered since 1964, 1975, and 1985, respectively. Since 2001, the Patient Register also includes outpatient physician visits in both private and public health care. Primary care is however not covered. The coverage is nearly complete for in-patient care, with a good validity for most diagnoses.[177] The coverage for outpatient care has gradually improved but is still somewhat lower, especially from private health care providers.[177, 178] Primary and secondary diagnoses are reported using a Swedish adaption of the International Classification of Diseases (ICD).

Cause of Death Register

The Cause of Death Register at the National Board of Health and Welfare contains information on dates and causes of death since 1961. Deaths of all Swedish citizens are recorded irrespective of whether they occurred in Sweden or abroad. It has been estimated that 93% of all deaths are reported within 10 days, and 100% within 30 days.[179] Deaths are recorded with underlying and contributing causes using ICD codes.

4.2.3.3 Definitions of prevalent and incident cases

Diabetes

Information from the National Diabetes Register was combined with information from the National Patient Register (primary and secondary diagnoses) to define prevalent and incident cases of diabetes. The register data was further complemented with self-reported information on prevalent diabetes from the 1997 and 2008 questionnaires. **Figure 4.5** illustrates how the three sources of information overlap and complement each other before and after the baseline in **paper III** and **paper IV** (1 January 1998).

The first date from either of the two registers was used as an approximation of date of diabetes diagnosis. In addition to the registered visit dates, we also considered the year of diabetes debut retrospectively reported in the Diabetes Register (date manually set to 1 July). Among the cohort participants registered in the Diabetes Register, information on debut year was available for 84%. Overall, 40% of the diagnosis dates were derived from the backdated Diabetes Register data, 19% from the first Diabetes Register record, and 41% from the first Patient Register record.

The registered data on diabetes type was somewhat inconsistent. About 14% of the cohort participants with diabetes had information on type that differed either within or between the two registers. We assigned the type with priority given to the Diabetes Register's epidemiological classification, followed by the clinical classification. For participants not registered in the Diabetes Register, and for those whose diabetes type was too inconsistently reported between visits, information from the Patient Register was used (ICD-10 code E10 for type 1, E11 for type 2, E12 for malnutrition-related diabetes mellitus, E13 for other specified diabetes mellitus, and E14 for unspecified diabetes mellitus).

In **paper III**, prevalent cases of diabetes within the COSM were excluded from the study population. Exclusion was made for any type of diabetes with diagnosis date before 1 January 1998 according to register data, as well as for self-reported prevalent diabetes on the 1997 questionnaire. Among 962 participants with pre-baseline diabetes according to self-report only, 530 were later registered in at least one of the two registers. Incident cases during follow-up through December 2012 were identified using the definition of type 2 diabetes and date of diagnosis described above. **Figure 4.6** illustrates how the two registers contributed to the case identification year by year during follow-up. In total, 49% of the incident cases were

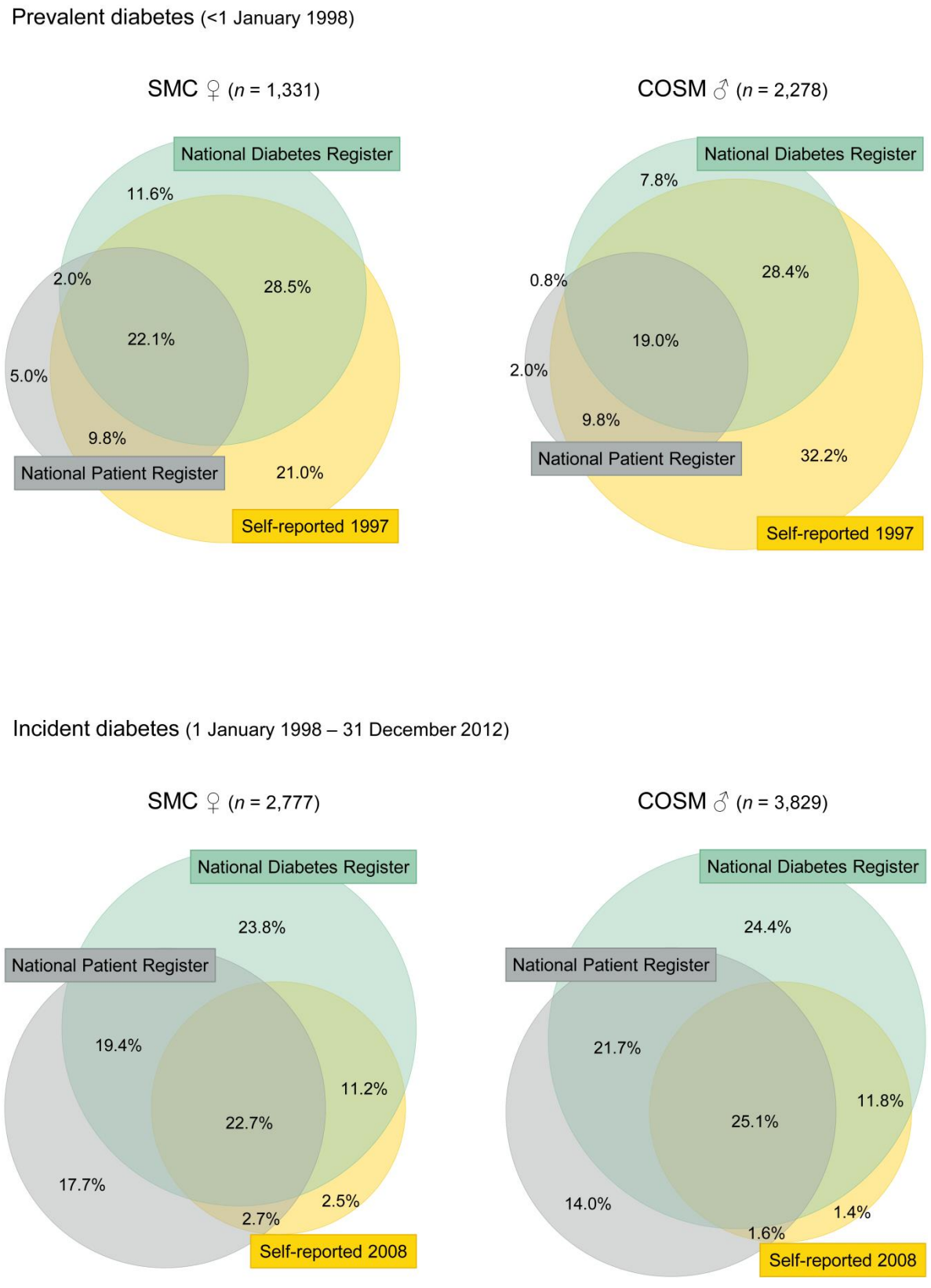


Figure 4.5 Overlap between the sources of diabetes information. Percentages of the total number of cohort participants with diabetes according to any source, after non-diabetes-related exclusions. For incident cases, numbers are calculated after exclusion of prevalent cases identified from any source. *SMC*, Swedish Mammography Cohort; *COSM*, Cohort of Swedish Men

recorded in both registers, 38% in the Diabetes Register only, and 13% in the Patient Register only. The date of diabetes diagnosis was derived from the backdated Diabetes Register data for 39%, from the first Diabetes Register record for 34%, and from the first Patient Register record for 27% of the incident cases.

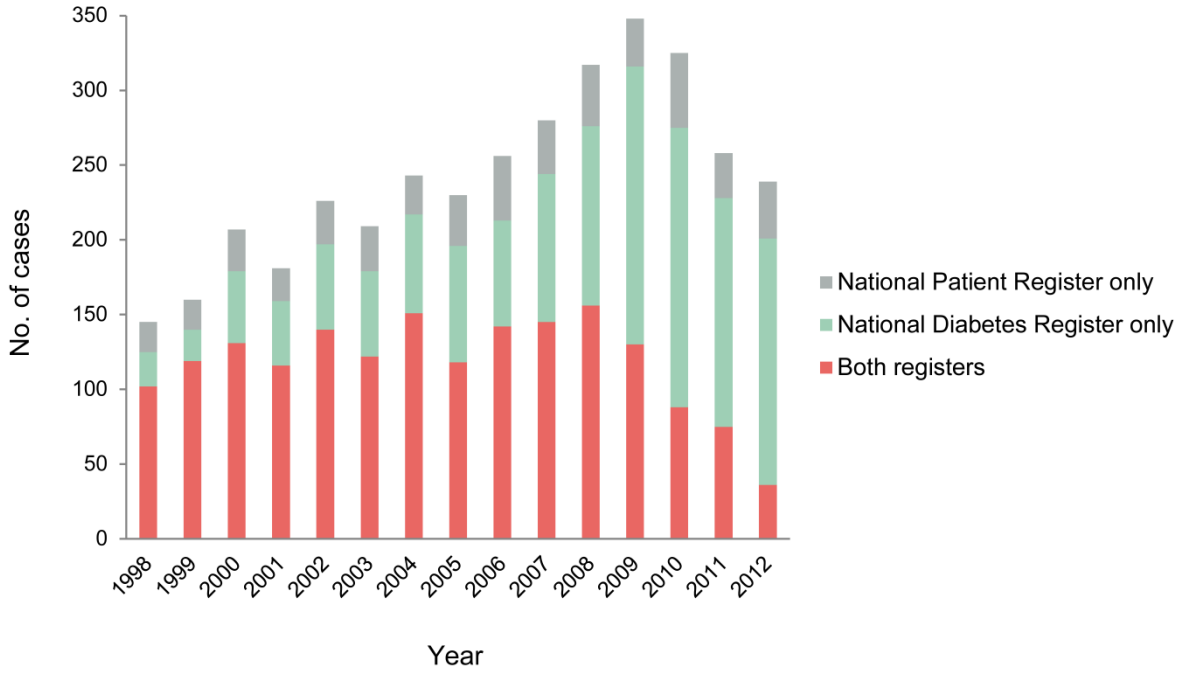


Figure 4.6 Sources of case identification during follow-up in **paper III**

In **paper IV**, only prevalent cases of type 2 diabetes were included in the study population. This selection was based on the definition of diabetes type and date of diagnosis described above. Self-reported diabetes was not considered because no information on type of diabetes or diagnosis date was available from the questionnaires. Among the 2,225 included participants, 82% were identified in both registers, 10% in the Diabetes Register only, and 8% in the Patient Register only. An additional number of 1,323 cohort participants had self-reported prevalent diabetes on the 1997 questionnaire but no registered diabetes diagnosis at baseline and were thus not included. Among these, 38% were identified in at least one of the two registers at a later date.

Cardiovascular disease

In **paper III** and **paper IV**, participants with cardiovascular disease before baseline were excluded. Exclusions were made for primary or secondary diagnoses with ICD-10 codes I20–I25 (ischemic heart diseases), 163 (cerebral infarction), I61 (intracerebral hemorrhage), I60 (subarachnoid hemorrhage), or I64 (stroke, not specified as hemorrhage or infarction) recorded in the Patient Register before 1 January 1998.

In **paper IV**, incident cases of MI were identified using ICD-10 code I21 (acute myocardial infarction) listed as primary diagnosis in the Patient Register, or as underlying cause of death in the Cause of Death Register. For stroke identification, primary diagnosis in the Patient Register or underlying cause of death in the Cause of Death Register with ICD-10 codes 163 (cerebral infarction), I61 (intracerebral hemorrhage), I60 (subarachnoid hemorrhage), or I64 (stroke, not specified as hemorrhage or infarction) were used.

Mortality

For the analysis of mortality endpoints in **paper IV**, deaths occurring during follow-up were ascertained through the Cause of Death Register. Coronary heart disease (CHD)-related deaths were defined as those listed with ICD-10 codes I20–I25 (ischemic heart diseases) as underlying cause.

4.2.4 Statistical analysis

4.2.4.1 Validation

In **paper II**, Pearson product-moment correlations were used to measure the strength of the relationship between FFQ-based long-chain omega-3 fatty acid intake estimates and the concentration of these fatty acids in adipose tissue. We also evaluated how reported total and fatty fish consumption correlated with long-chain omega-3 fatty acids in adipose tissue by using Spearman's rank correlations. The fatty acid intake estimates were expressed as percentages of total fat intake for analogy with the adipose tissue content data. We used natural logarithmic transformations to improve normality. Correlations with individual and total long-chain omega-3 fatty acids were assessed. We further evaluated how earlier reports of dietary intake (FFQ-87 and FFQ-97) and long-term average intake (mean of estimates from two or three FFQ assessments) correlated with adipose tissue content. Finally, the influence of body fatness was evaluated by means of stratified correlation analysis and linear regression analysis with an interaction term between FFQ-based total long-chain omega-3 fatty acid intake and body fat percentage.

4.2.4.2 Survival analysis

In **paper III** and **paper IV**, we used Cox proportional hazards regression models to estimate hazard ratios (HRs) with 95% CIs for the associations of interest. Age was used as the underlying time scale in all analyses, and participants contributed person-time from 1 January 1998 until the date of the diagnoses of interest, death, or end of follow-up (31 December 2012). We assessed the associations by categories of total fish consumption as well as the three individual finfish items, shellfish, and fried fish. Trends across categories were tested by assigning each participant the median value in their respective exposure category and modeling this as a continuous variable. The proportional hazards assumption was tested with the Schoenfeld residual test, with no violations indicated.

All multivariable analyses were conducted adjusting for BMI, physical activity, education, cigarette smoking, total energy intake, intake of alcohol, and the DASH diet component score. In **paper IV**, the models additionally included time since diabetes diagnosis, history of high cholesterol, and history of hypertension; further adjustment for family history of MI before the age of 60 years, aspirin use, or fish oil supplement use did not change the associations and these variables were thus not included. Individual fish and shellfish items were mutually adjusted in all analyses. Missing values for covariates were treated as separate categories in the models.

In **paper III**, we presented results from an additional multivariable model with further adjustment for dietary exposure to PCBs and MeHg. To explore the potential impact of adjustment for collinear variables, we also examined the association between total fish consumption and type 2 diabetes risk across strata of dietary PCB and MeHg exposure (median splits). The dose–response relationship between fried fish consumption and type 2 diabetes incidence was examined using restricted cubic splines with knots at 10, 50, and 90% of the distribution.

As **paper IV** included both women and men, we assessed potential interaction by sex using the likelihood ratio test to compare models with and without a cross-product term. Sensitivity analyses excluding cases that were identified during the first two years of follow-up were conducted in both **paper III** and **paper IV**. In **paper III** we further evaluated the impact of excluding participants who reported any use of fish oil supplements. We also performed analyses of total fish consumption using an alternative reference category of non-consumers.

5 RESULTS

5.1 META-ANALYSIS ON TYPE 2 DIABETES INCIDENCE

5.1.1 Literature search

The literature search up to 15 December 2011 identified 2,474 articles, among which 21 were obtained for full-text review. Among these, five articles were excluded because of non-eligible exposure and/or outcome definition, and two were outdated by subsequent publications on the same study populations. A total of fourteen publications were thus included in the meta-analysis.[6-15, 160, 180-182]

5.1.2 Study characteristics

The fourteen included articles were published between 2001 and 2011, and reported results from sixteen separate cohort studies. Altogether, these involved 527,441 study participants and 24,082 cases of diabetes. Thirteen of the studies reported results on fish consumption and thirteen on intake of long-chain omega-3 fatty acids. Seven studies were conducted in the US,[7, 8, 12, 13, 181] four in Europe,[6, 9, 10, 180, 182] four in Asia,[11, 14, 15] and one in Australia.[160]

All studies used self-administered or interviewer-administered food frequency questionnaires (FFQs) to collect dietary data. The exposure information was updated during follow-up in six of the studies.[8, 12, 15] All but three studies[6, 10, 12] used self-reports to identify diabetes cases. In some of these studies the self-reported information was followed up with confirmation from other sources.[8, 9, 13, 160, 180, 182] The most frequently included covariates in the multivariable analyses were age, sex, energy intake, smoking, body mass index (BMI), physical activity, and alcohol intake. Thirteen of the studies reached a quality score of at least six stars out of nine on the Newcastle-Ottawa Quality Assessment Scale.[6, 8-12, 14, 15, 180-182]

5.1.3 Summary associations

For both fish consumption and dietary intake of long-chain omega-3 fatty acids, the results across studies were mixed, ranging from inverse to direct associations. Because of a high degree of statistical heterogeneity (fish, I^2 = 81.3%; p = 0.001; long-chain omega-3 fatty acids, I^2 = 78.3%; p = 0.001), we did not combine results into overall summary risk estimates. While sex, self-administered or interviewer-administered FFQ, study quality, or method for case ascertainment did not appreciably explain heterogeneity (**Table 2, paper I**), there were substantial differences according to geographical region (USA, Europe, Asia/Australia).

The estimated relative risks (RR) for type 2 diabetes associated with each serving per week increment in total fish consumption are shown in **Figure 5.1**, for individual studies and combined by geographical region. The summary RRs were 1.05 (95% CI 1.02–1.09) for studies conducted in the US, 1.03 (95% CI 0.96–1.11) for studies conducted in Europe, and 0.98 (95% CI 0.97–1.00) for Asian studies.

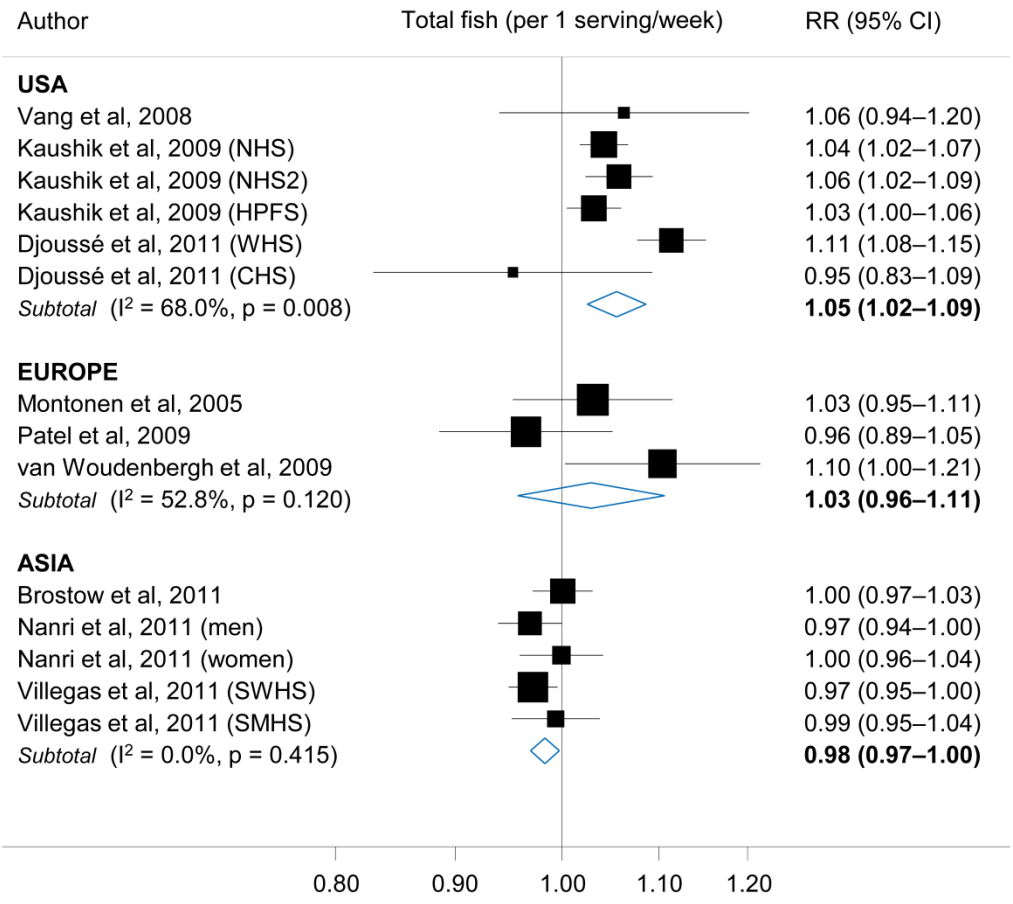


Figure 5.1 Relative risks with 95% confidence intervals for type 2 diabetes, for each serving per week increment in total fish consumption

For dietary intake of long-chain omega-3 fatty acids, each 0.30 gram per day increment was associated with RRs of 1.17 (95% CI 1.09–1.26), 0.98 (95% CI 0.70–1.37), and 0.90 (95% CI 0.82–0.98) in US, European, and Asian/Australian studies respectively. No evidence of publication bias was detected using the Egger test (fish, p = 0.85; long-chain omega-3 fatty acids, p = 0.10). We found no evidence of departure from linearity (fish, $p_{nonlinearity}$ = 0.81; long-chain omega-3 fatty acids, $p_{nonlinearity}$ = 0.76).

5.1.4 Sensitivity analyses

Both the summary RR estimates and the measures of statistical heterogeneity remained largely similar in all sensitivity analyses. After omission of one study at the time, the overall I^2 ranged from 70.2% to 82.8% for fish consumption, and from 73.2% to 80.1% for long-chain omega-3 fatty acid intake. A high degree of heterogeneity remained also after changing the fish serving size from 100 grams to 80 grams (I^2 = 82.5%), or to 140 grams (I^2 = 78.9%). All US studies reported fish consumption in servings and were thus unaffected. The summary RRs in European and Asian studies for each serving per week increment were 1.02 (95% CI 0.97–1.09) and 0.99 (95% CI 0.98–1.00) using 80 grams, and 1.04 (95% CI 0.94–1.14) and 0.98 (95% CI 0.96–1.00) using 140 grams. Omission of three nested case-control studies from the meta-analysis of long-chain omega-3 fatty acid intake also had little impact, with the overall heterogeneity remaining high (I^2 = 79.7%).

5.1.5 Updated meta-analyses

In an updated literature search up to 29 February 2016, five new eligible studies on fish consumption published after **paper I** were identified (including **paper III**). All of these were conducted in European populations.[17-19 16, 183] In an updated meta-analysis incorporating the new findings, the statistical heterogeneity in the overall summary estimate remained (I^2 = 76.7%; p = 0.001). The null association in European studies also remained, with a summary RR for type 2 diabetes of 1.01 (95% CI, 0.99–1.02) for each serving per week increment in total fish consumption (**Figure 5.2**).

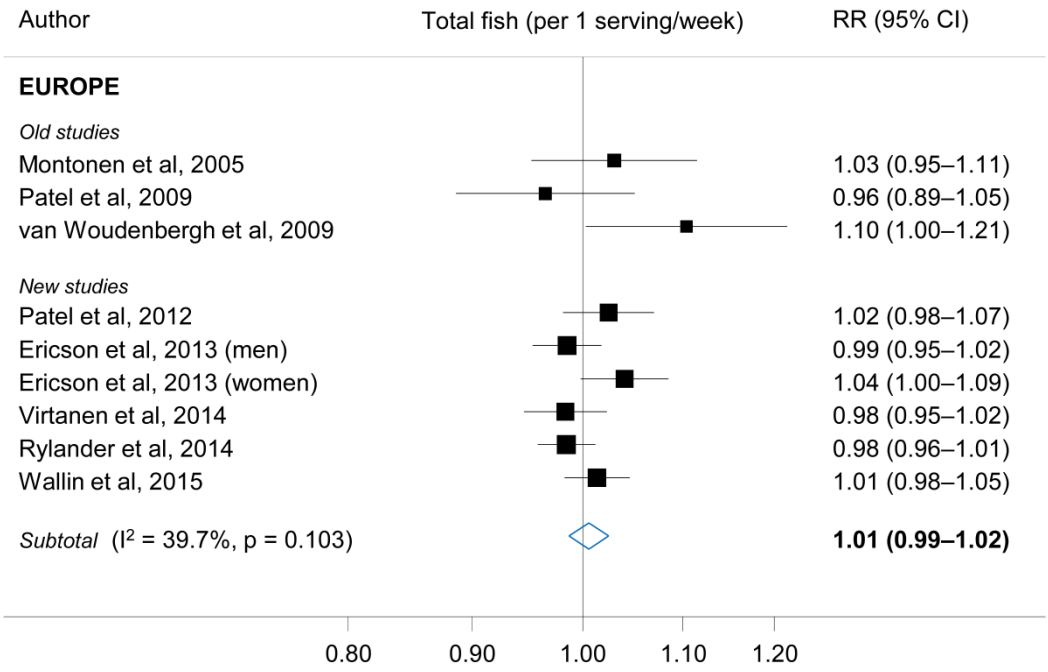


Figure 5.2 Updated meta-analysis of European studies published through February 2016, including five new studies published during 2012–2015. Relative risks with 95% confidence intervals for type 2 diabetes, for each serving per week increment in total fish consumption.

With the five new publications, the number of studies on different types of fish allowed for meta-analytic summary of fatty fish, lean fish, and shellfish consumption in relation to type 2 diabetes risk. The results are presented both overall and separately for studies conducted in Asia and Europe. No US studies reported results by types of fish.

There was no association with neither fatty (**Figure 5.3**) nor lean fish (**Figure 5.4**). No statistically significant heterogeneity was observed in the overall summary estimates, although results on lean fish were heterogeneous within Europe.

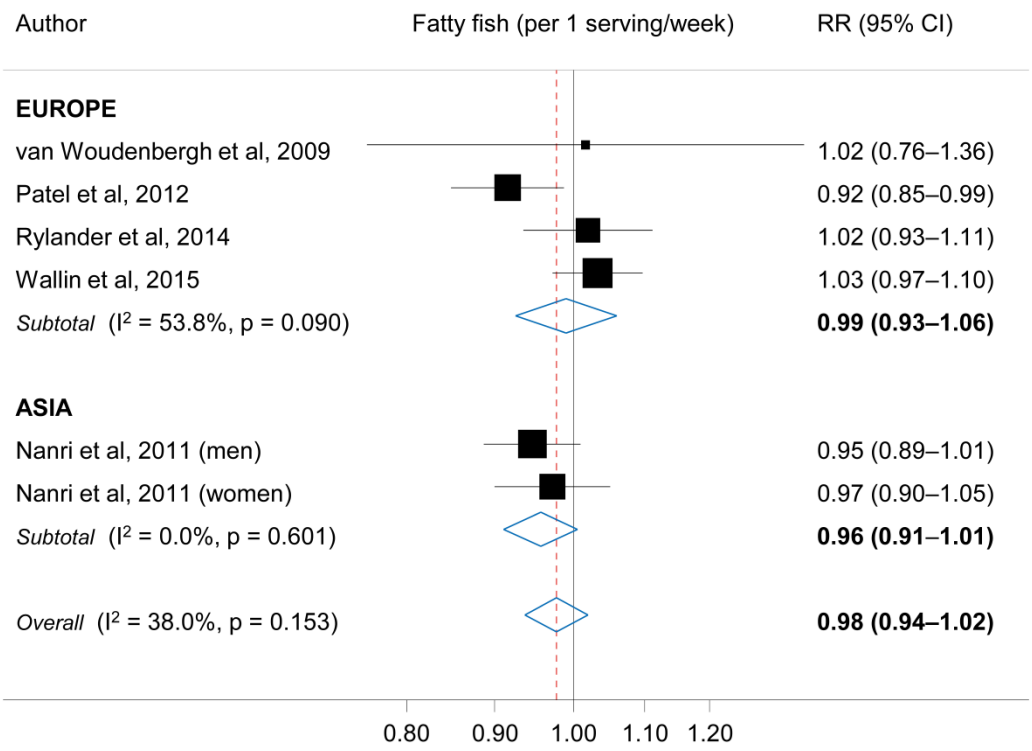


Figure 5.3 Relative risks with 95% confidence intervals for type 2 diabetes, for each serving per week increment in fatty fish consumption. Updated meta-analysis including studies published through February 2016.

Results on shellfish differed substantially between regions (I^2 = 77.1%; p = 0.001), with an inverse association with type 2 diabetes risk in Asian studies, and a direct association in European studies (**Figure 5.5**). The total number of studies on shellfish is however still limited.

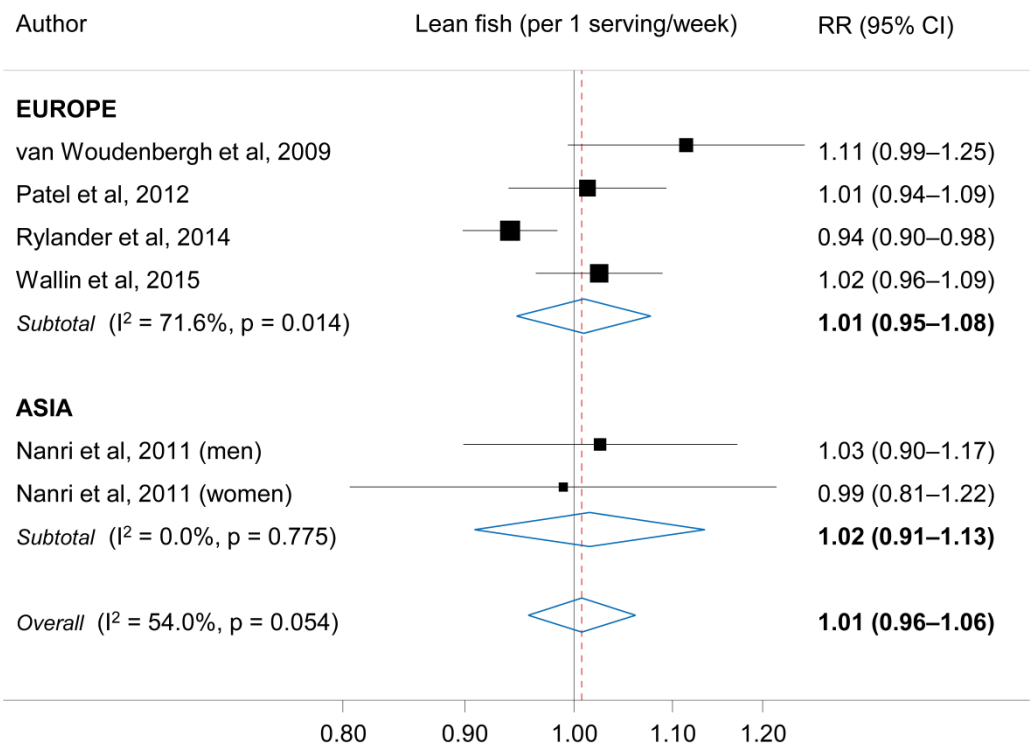


Figure 5.4 Relative risks with 95% confidence intervals for type 2 diabetes, for each serving per week increment in lean fish consumption. Updated meta-analysis including studies published through February 2016.

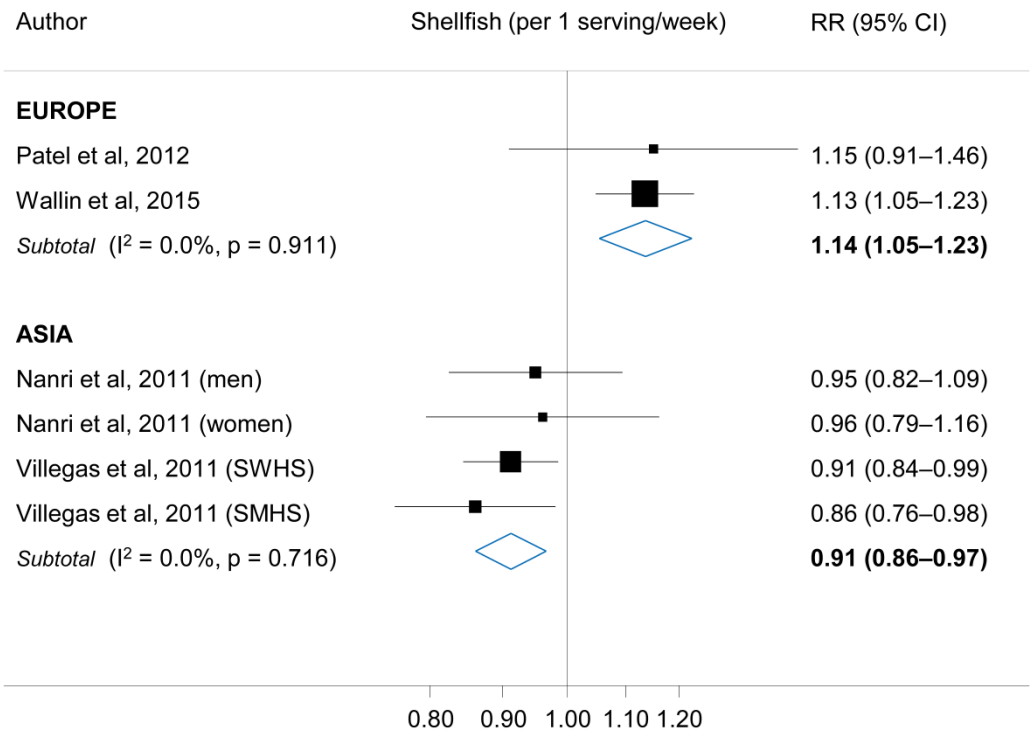


Figure 5.5 Relative risks with 95% confidence intervals for type 2 diabetes, for each serving per week increment in shellfish consumption. Updated meta-analysis including studies published through February 2016.

5.2 VALIDITY OF QUESTIONNAIRE-BASED EXPOSURE ESTIMATES

Comparing estimates from FFQs filled in by the 239 women included in **paper II**, the mean fish consumption was somewhat higher in 2003 than in 1997. This owed to an increase in reported fatty fish consumption from 1.2 (±1.3) servings per week to 1.6 (±1.9) servings per week, whereas lean fish consumption decreased marginally from 0.9 (±1.2) servings per week to 0.8 (±0.7) servings per week. In FFQ-03, fish and seafood items (including caviar) collectively contributed to 77% of total long-chain omega-3 fatty acid intake (**Figure 5.6**).

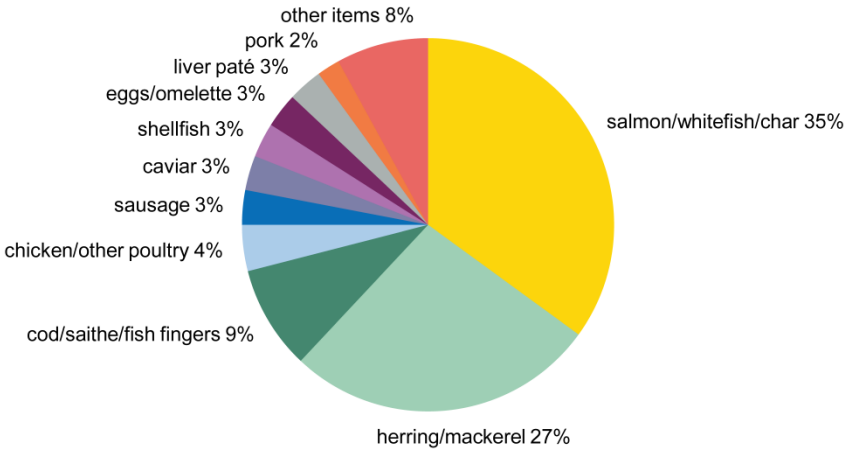


Figure 5.6 Major sources of dietary long-chain omega-3 fatty acid intake according to FFQ-03

Pearson correlation coefficients for FFQ-based estimates of total and individual long-chain omega-3 fatty acid intake in comparison with their concentrations in adipose tissue are presented in **Table 5.1**. Validity was assessed as correlations for the estimates from FFQ-03, as it queried diet in the time frame expected to be reflected in adipose tissue. Significant correlations, although lower in strength, were observed also for long-term intake estimates derived from past FFQs. Using the mean intake from two or three FFQ assessments generally enhanced the correlations (**Table 2, paper II**).

Table 5.1 Correlations between relative dietary intake (% of total fat intake) from three food frequency questionnaire assessments over 15 years and adipose tissue content in 239 women

	FFQs *vs.* adipose tissue (mean time before adipose tissue sampling)		
	FFQ-03 (1.8 months)	FFQ-97 (6.5 years)	FFQ-87 (14.9 years)
Total long-chain omega-3 fatty acids	0.41	0.29	0.31
EPA	0.32	0.21	0.21
DPA	0.29	0.22	0.21
DHA	0.48	0.33	0.34

Data are presented as Pearson correlation coefficients based on $\log_e$-transformed values. *DHA*, docosahexaenoic acid; *DPA*, docosapentaenoic acid; *EPA*, eicosapentaenoic acid; *FFQ*, food frequency questionnaire

We further performed a stratified analysis for total long-chain omega-3 fatty acids by tertiles of body fat percentage (data available for 233 women). The correlation between dietary intake estimated by FFQ-03 and adipose tissue content was lower among participants in the highest body fat percentage tertile (**Table 3, paper II**). A statistically significant interaction between dietary intake and body fat percentage in predicting adipose tissue content was detected using linear regression ($p_{interaction} = 0.003$).

Spearman correlation coefficients for the FFQ-03 estimates of total and fatty fish consumption in comparison with concentrations of long-chain omega-3 fatty acids in adipose tissue are presented in **Table 5.2**. Fish consumption was correlated with the individual fatty acids in adipose tissue according to the same pattern as observed on the nutrient level, i.e., highest correlation with docosahexaenoic acid (DHA) and lowest with docosapentaenoic acid (DPA).

Table 5.2 Correlations between fish consumption estimates from FFQ-03 and adipose tissue content of total and individual long-chain omega-3 fatty acids in in 239 women

	FFQ-03 total fish	**FFQ-03** fatty fish
Adipose tissue		
Total long-chain omega-3 fatty acids	0.29	0.31
EPA	0.27	0.29
DPA	0.18	0.19
DHA	0.34	0.36

Data are presented as Spearman correlation coefficients. Total fish is the sum of herring/mackerel, salmon/ whitefish/char, and cod/saithe fish fingers; fatty fish is the sum of herring/mackerel and salmon/whitefish/char. *DHA*, docosahexaenoic acid; *DPA*, docosapentaenoic acid; *EPA*, eicosapentaenoic acid; *FFQ*, food frequency questionnaire

5.3 FISH CONSUMPTION IN RELATION TO TYPE 2 DIABETES INCIDENCE

Among the men followed from 1 January 1998 up to 31 December 2012, we identified 3,624 incident cases of type 2 diabetes. Baseline characteristics of the study participants are presented in **Table 1, paper III.**

We observed no association between total fish consumption in the primary multivariable model adjusting for BMI, physical activity, education, cigarette smoking, alcohol, and dietary factors. Compared with men who consumed less than one serving per week, the hazard ratio (HR) for those who consumed four servings per week or more was 1.00 (95% CI 0.85–1.18). Further adjustment for dietary exposure to polychlorinated biphenyls (PCBs) and methylmercury (MeHg) had a marked impact on the risk estimates although the association remained statistically nonsignificant (HR = 0.79, 95% CI 0.60–1.04; **Figure 5.7**).

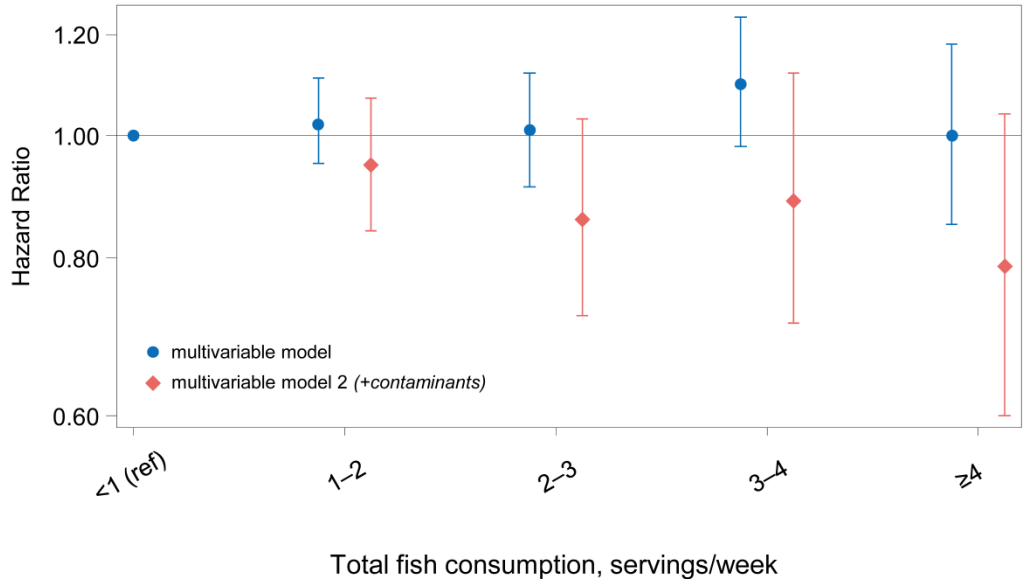


Figure 5.7 Association between fish consumption and risk of type 2 diabetes, in multivariable analyses before and after adjustment for dietary exposure to polychlorinated biphenyls and methylmercury

Fried fish consumption was associated with higher risk of type 2 diabetes, without a marked impact of additional adjustment for dietary contaminant exposures (**Table 3, paper III**). In a restricted cubic spline model, we found no evidence of a nonlinear association ($p_{nonlinearity}$ = 0.21). The dose–response relationship is shown in **Figure 5.8**. Each serving per week increment in fried fish consumption was associated with a HR of 1.07 (95% CI 1.02–1.11).

None of the finfish items were individually associated with type 2 diabetes risk, whereas a higher risk was observed for shellfish consumption (≥1 serving per week *vs.* never/seldom; HR = 1.21, 95% CI 1.07–1.36). The associations for individual fish and shellfish items, before and after adjustment for dietary contaminant exposures, are shown in **Figure 5.9**.

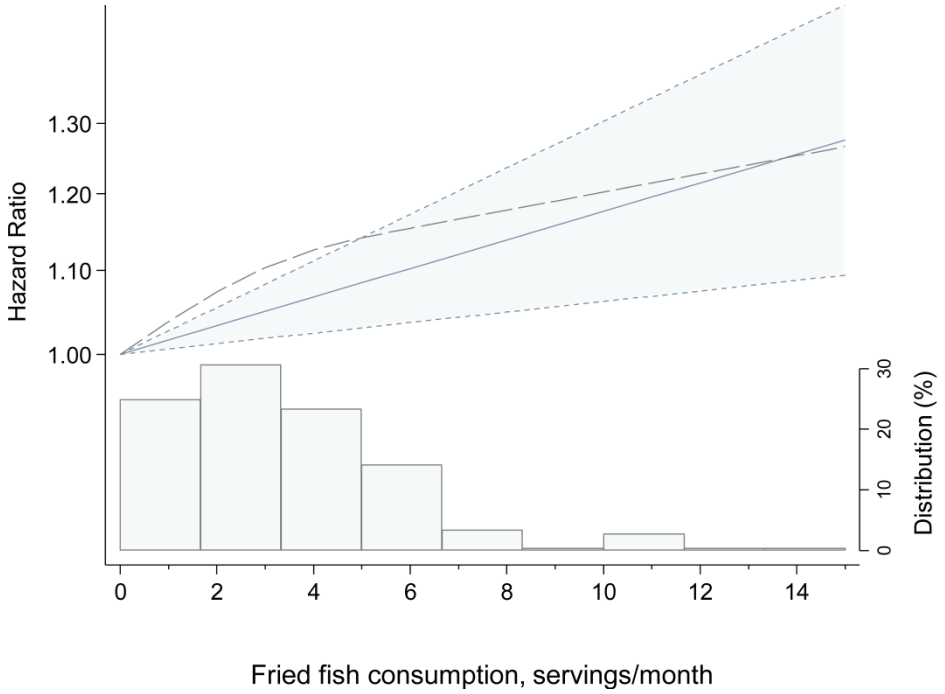


Figure 5.8 Linear dose–response hazard ratio (solid line) with 95% confidence intervals (short dashed lines) and hazard ratio estimated with a restricted cubic spline model (long dashed line) for the association between fried fish consumption and risk of type 2 diabetes. The histogram represents the distribution of fried fish consumption.

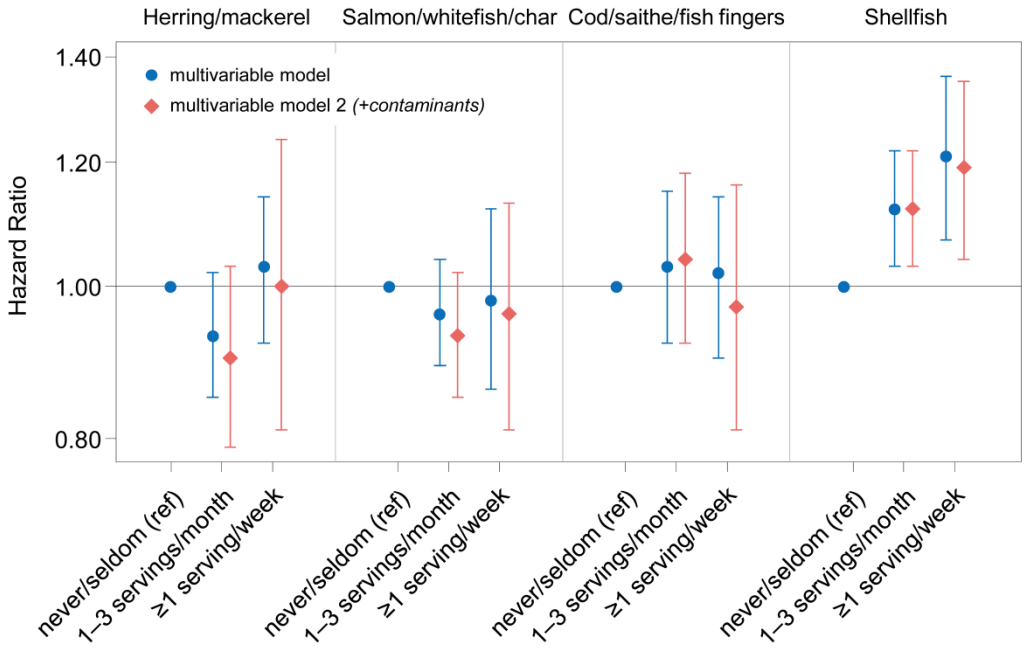


Figure 5.9 Associations between consumption of individual fish items and risk of type 2 diabetes, in multivariable analyses before and after adjustment for dietary exposure to polychlorinated biphenyls and methylmercury

To assess the potential impact of misclassification of prevalent cases as incident, we performed sensitivity analyses excluding type 2 diabetes cases identified during the first two years of follow-up (*n* = 305). We further evaluated the associations after exclusion of fish oil supplement users (*n* = 1,649; 178 cases). The results were not markedly influenced by these exclusions, neither before nor after adjustment for dietary exposure to PCBs and MeHg. In an additional sensitivity analysis using only non-consumers as the reference group in the analysis of total fish consumption (*n* = 911; 2.6%), similar results were also observed both in the primary model and in the contaminant-adjusted model.

As the dietary contaminant exposure estimates were relatively highly correlated with total fish consumption (PCBs, r = 0.77; MeHg, r = 0.70), we further explored the potential impact of the adjustment for collinear variables. The association between total fish consumption and risk of type 2 diabetes was fairly similar across strata of dietary PCB and MeHg exposures, supporting that collinearity did not produce spurious results in multivariable-adjusted model including these contaminants.

5.4 FISH CONSUMPTION IN RELATION TO CARDIOVASCULAR DISEASE AND MORTALITY AMONG PEOPLE WITH TYPE 2 DIABETES

Among the 2,225 women and men in the cohorts with a diabetes diagnosis before baseline (1 January 1998), we identified 333 incident cases of myocardial infarction (MI) and 321 incident cases of stroke during follow-up through 31 December 2012. During the same period, 771 deaths occurred, of which 154 were registered with coronary heart disease (CHD) as underlying cause. The baseline characteristics of the study participants are presented in **Table 1, paper IV**.

All results are presented for women and men combined as no statistically significant interactions by sex were observed. The associations between total fish consumption and incident MI and stroke, as well as total and CHD-related mortality are shown in **Figure 5.10**.

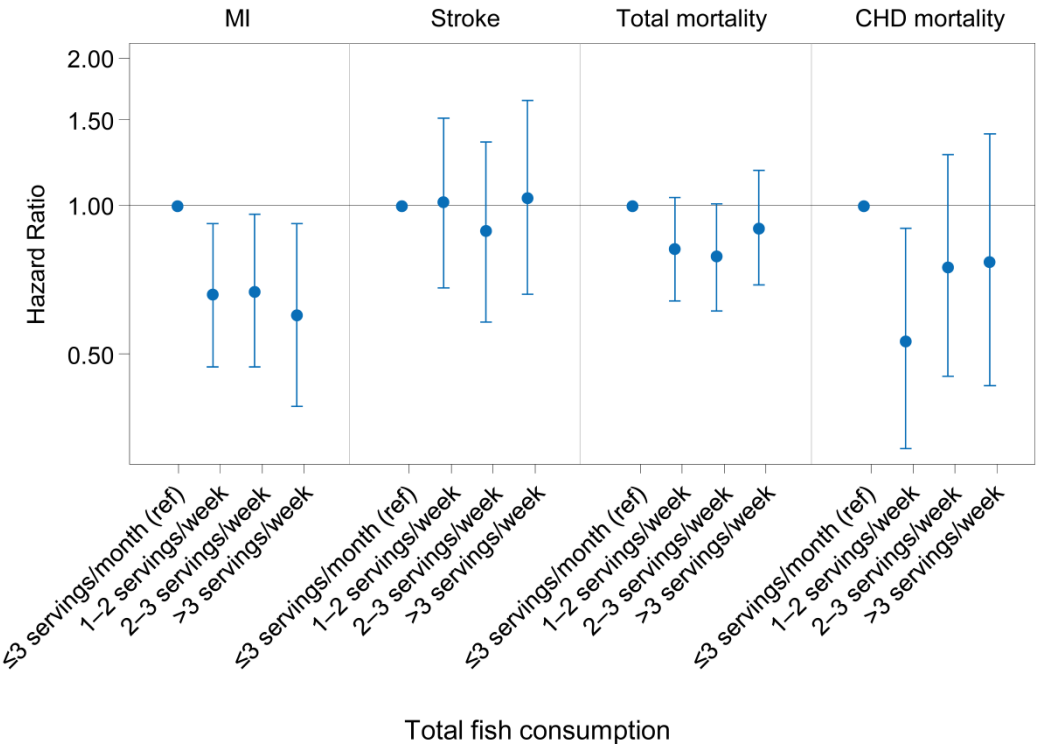


Figure 5.10 Associations between total fish consumption and risk of myocardial infarction (MI) and stroke, and total and coronary heart disease (CHD)-related mortality among women and men with prevalent type 2 diabetes

Total fish consumption was inversely associated with MI, with lower risk observed already at modest fish consumption levels. Compared with those who consumed no more than three servings per month, the multivariable-adjusted HRs were 0.66 (95% CI 0.47–0.92), 0.67 (95% CI 0.47–0.96), and 0.60 (95% CI 0.39–0.92) for those who consumed one to two, two to three, and more than three servings per week, respectively. No association was observed with stroke. For total mortality, a modest inverse association was indicated but not statistically significant. The inverse association with CHD-related mortality was significant

only at the level of one to two servings per week compared with lower consumption (HR = 0.53, 95% CI 0.32–0.90).

Among the individual fish items, herring/mackerel consumption was associated with lower risk of MI (≥1 serving per week *vs.* <1 serving per month, HR = 0.70, 95% CI 0.50–0.98), whereas there were no associations with salmon/whitefish/char or shellfish. Consumption of one to three servings per month of cod/saithe/fish fingers was also inversely associated with MI (HR = 0.64, 95% CI 0.46–0.90), whereas the association was not statistically significant at higher consumption levels (**Table 2, paper IV**). In relation to the mortality outcomes, inverse associations were observed only for one to three servings per month of cod/saithe/fish fingers (**Table 3, paper IV**).

For MI as well as for the mortality outcomes, the associations with fried fish were largely in line with what we observed for total fish. Compared with those in the lowest tertile of fried fish consumption, the multivariable HRs for those in the highest tertile were 0.69 (95% CI 0.51–0.93) for MI, 0.91 (95% CI, 0.75–1.10) for total mortality, and 0.74 (95% CI 0.47–1.15) for CHD-related mortality. For stroke, the association with fried fish differed more from what was observed for total fish, but was still not statistically significant (HR = 1.25, 95% CI 0.93–1.67).

Sensitivity analyses excluding incident cases or deaths identified during the first two years of follow-up (MI: 49 cases; stroke: 34 cases; total mortality: 29 cases; CHD-related mortality: 8 cases), had no marked impact on the results.

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

Observational epidemiology is associated with limitations and potential sources of error that are important to consider. Issues of relevance for the papers included in this thesis and their potential influence on the results are discussed in the following section.

6.1.1 Random error

Random error is the variability in the data due to chance and relates to the *precision* of the estimation. The amount of random error in an estimate can be reduced, and thus precision enhanced, by increasing the sample size. In cohort studies, the limiting quantity is usually the number of cases.[184]

In the studies included in this thesis, precision is indicated using 95% confidence intervals (CIs). A narrow interval implies high precision of the point estimate. Formally, if the interval does not include the null value (a hazard ratio of 1.00) the association is considered to be statistically significant. The lower the strength of the association, the higher the precision needs to be to be able to reject a null hypothesis of no association. The probability to correctly reject the null hypothesis is called the *statistical power*.[184] Given that most diet–disease associations are relatively modest in strength, and that variation is augmented by inevitable measurement errors in dietary assessments, studies of such associations require large sample sizes.

In **paper III**, the large number of incident type 2 diabetes cases (n = 3,624) provided high statistical power. Although the data indicated an inverse association after adjustment for dietary contaminant exposures, the null hypothesis of no association could however not be formally rejected. **Paper IV** included a smaller number of cases for all outcomes. The results for myocardial infarction (MI) were however statistically significant despite relatively few cases (n = 333). The analyses of coronary heart disease (CHD)-related deaths (n = 154) would have benefited from higher statistical power.

It is important to note that the formal interpretation of the CI applies under the assumption of no systematic errors, and thus relates only to chance as an alternative explanation for the findings. In view of the risk of non-chance related alternative explanations, evaluation of associations solely based on the arbitrary statistical significance dichotomy should be avoided. The strength and the precision of an association should always be considered in context of previous evidence, biological plausibility, and potential bias.

6.1.2 Systematic error

Systematic error refers to the type of errors that would remain even if the study was infinitely large. Such errors undermine the *internal validity* of a study, as they can distort results by either underestimation or overestimation of associations. Violations of internal validity can be broadly grouped into three major types of bias: selection bias, information bias, and confounding bias.[184]

6.1.2.1 Selection bias

Selection bias arises when participation in the study is related to both exposure and outcome status, which results in an association between exposure and outcome that differ between those who participated in the study and those who were eligible but did not participate. Such bias can originate both from the procedures of recruitment into the study and from differential completeness of follow-up.[184]

In prospective cohort studies selection bias related to recruitment is generally prevented, as selection into the study cannot be dependent on an outcome that has not yet occurred. Bias related to non-complete follow-up may on the other hand be an issue, if the degree of follow-up is related to exposure status. In **paper III** and **paper IV**, we aimed to minimize loss to follow-up by linkage of the Swedish personal identity number to national registers. The high coverage of the Cause of Death Register as well as of in-patient care in the National Patient Register enabled virtually complete follow-up in **paper IV**. Follow-up for type 2 diabetes is more challenging as the Patient Register does not cover primary care. As illustrated in **Figure 4.4** and **Figure 4.5**, the National Diabetes Register was the most important source for case ascertainment in **paper III**, but the combined use of the two registers was necessary for more comprehensive identification. The Diabetes Register currently reaches nearly complete coverage in the study area, but coverage was markedly lower during the earlier years of follow-up. Identification of early cases was substantially enhanced by the retrospective recording of diabetes debut year, but we have inevitably missed some cases that died before the coverage of the register improved. This loss to follow-up would result in selection bias only if it was related to fish consumption.

Differential loss to follow-up may have affected some of the studies included in **paper I** because of case ascertainment that depended on self-reports. It is possible that less health-conscious participants are less interested in completing additional questionnaires, which could lead to different response rates among high and low consumers of fish.

6.1.2.2 Information bias

Information bias is introduced by errors in measurements of the variables of interest. Measurement error for discrete variables is often referred to as *misclassification*, and can be further divided into *differential* or *nondifferential*. Misclassification of exposure is differential if it differs depending on outcome status and nondifferential if it is independent of outcome

status. The same applies for misclassification of outcome in relation to exposure status. Differential misclassification can bias the results in either direction and thus produce spurious associations between exposure and outcome. Nondifferential misclassification of binary variables results in underestimation of the strength of the association. For variables with more than two levels, results may also be biased away from the null.[184]

Misclassification of exposure

Dietary assessment is inevitably subject to some degree of misclassification, both because of normal within-person variation and the difficulties in correctly recalling and reporting dietary habits.

In **paper II**, we aimed to assess the measurement error in the exposure estimates used in **paper III** and **paper IV**. Reasonable validity in the food frequency questionnaire (FFQ)-based estimates was indicated by correlation coefficients of 0.4 for total long-chain omega-3 fatty acid intake and 0.3 for total and fatty fish consumption. Correlations with dietary intake reported as long as fifteen years prior to adipose tissue sampling further support that the FFQ-estimates can reflect intake over longer periods of time. Although the use of an objective biomarker is a strength, it should be noted that the correlations reflect a *relative validity* as the adipose tissue content of long-chain omega-3 fatty acids is not a perfect reflection of actual dietary intake. In addition, biomarker measurements can also be subject to misclassification due to factors related to sampling, storage, laboratory measurement errors, as well as biological within-person variation.

In the original prospective studies of fish consumption (**paper III** and **paper IV**) as well as those included in the meta-analysis (**paper I**), all participants were free of the studied diseases when exposure information was collected. This prospective design prevents differential misclassification as the correctness of self-reports should not be dependent on a future outcome. However, the uncertainty in the register-derived date of diabetes diagnosis may have resulted in misclassification of some prevalent cases as incident in **paper III**, which in turn could have contributed to some differential misclassification of exposure (as discussed below under *misclassification of outcome*). Nondifferential misclassification of exposure has inevitably affected **paper III**, **paper IV**, and the studies included in **paper I** to some degree, which would most likely have attenuated the associations. Bias away from the null can however not be excluded because of the polychotomous exposure.

Misclassification of outcome

Because of the slow onset and vague initial symptoms, we can expect that a number of cohort participants who developed type 2 diabetes during follow-up in **paper III** remained undiagnosed. The tendency of underdiagnosis is most likely independent of fish consumption, and thus represents a nondifferential misclassification of a binary outcome that would bias the associations towards the null. However, we cannot exclude the possibility of differences in diabetes detection if fish consumption is related to an overall health

consciousness. It is possible that more health-conscious participants are more active in seeking medical care and therefore get diagnosed earlier. The opposite scenario is on the other hand also possible, i.e. that less health-conscious participants are likely to have a poorer health in general, resulting in greater medical attention and higher likelihood to have their diabetes detected and diagnosed.

The uncertainty in the register-derived date of diabetes diagnosis poses an additional potential problem as some prevalent cases may have been misclassified as incident in **paper III**. As dietary counseling is an integral part of diabetes management, the prevalent disease may have affected actual dietary habits and/or the correctness in reports of dietary habits. To limit such impact, we based the exclusion of prevalent cases on self-reports in addition to the register data. We further performed sensitivity analyses excluding cases that were identified during the first two years of follow-up. The virtually unaffected results indicate no substantial influence.

For the outcomes studied in **paper IV** (MI, stroke, and mortality), the National Patient Register and the Cause of Death Register are expected to provide virtually complete coverage and highly accurate information. Underascertainment of diabetes would however have influenced inclusion in the study population. This would be unfortunate because of the impairment of statistical power, but is unlikely to have biased the results.

6.1.2.3 Confounding

Confounding refers to distortion of results due to a factor that covaries with the studied exposure in the study population, and that is also independently associated with the studied disease. The effect of such a factor can in part or in whole explain an observed association (or lack thereof). To be defined as a confounder, the factor should not be an intermediate in the causal pathway between exposure and outcome, as a part of its effect in such a case would represent an effect of the exposure.[184]

In observational studies where exposures are not randomly distributed between groups, clustering of health related habits and lifestyle factors is likely to result in confounding if these factors are not appropriately controlled for in the design or in the statistical analysis. Fish consumption may be related to healthier behaviors in general, which has the potential to confound associations towards the inverse direction. To obtain valid risk estimates, identification of and adjustment for confounders is therefore a critical issue.

In the prospective studies included in this thesis (**paper III** and **paper IV** as well as studies included in **paper I**), confounding was controlled by restrictions and by inclusion of covariates in the statistical models. The studies included in **paper I** controlled for potential confounding to varying degrees. Factors such as age, sex, energy intake, smoking, body mass index (BMI), physical activity, and alcohol were included in most studies, while control for other factors varied substantially. There were however no apparent differences in results depending on the included covariates. In **paper III** and **paper IV**, several potentially

confounding factors, including demographic, medical, lifestyle and dietary factors seen as established or potential risk factors for the studied outcomes were taken into consideration. Confounding by age was accounted for as the time scale of the analyses. To account for diet, we used overall dietary quality as assessed by the DASH (Dietary Approaches to Stop Hypertension) score. An alternative approach with adjustment for individual dietary factors resulted in similar associations in all analyses.

In **paper III**, the second multivariable model included variables of dietary contaminant exposure. These factors cannot be considered to be confounders as their potential effect on the outcome would be a part of the effect of fish consumption. Inclusion of such factors in the model represents a theoretical "removal" of their effect to evaluate what effect of fish consumption would remain. A potential problem with this approach is collinearity, because of the relatively high correlations between the estimated contaminant exposures and fish consumption. Given that the CIs were not heavily inflated, this did not appear to be an issue of great concern. An approach to explore this further was to examine the association across strata of contaminant exposures, with fairly similar estimates supporting that collinearity did not produce spurious results. **Paper III** has an important strength in the large number of cases, allowing us to control for a large number of factors. In **paper IV**, we were not able to include the relatively highly correlated dietary contaminant exposure variables because of the considerably smaller study size.

It should be noted that covariates included in multivariable analyses also are subject to measurement error, which may lead to inadequate adjustment as some 'residual' confounding remains.[184] Most of the included covariates in **paper III** and **paper IV** are subject to the limitations of self-reported data. Because of a larger within-food variation for contaminants as compared to intrinsic components in food, the precision of the dietary contaminant exposure estimates may be further limited. Additional factors that are unknown or unmeasured may also have influenced the observed associations. Unmeasured factors of potential interest include family history of diabetes, use of medications, elevated blood glucose in the prediabetes range (**paper III**), measures of glycemic control or presence of other diabetes complications (**paper IV**), as well as aspects of diet not captured by the FFQ.

6.1.3 Generalizability

Generalizability, also called *external validity*, concerns the inference of results to an external population. Pursuit of representativeness to obtain generalizable results is however not necessarily a priority in studies of exposure–disease associations. Restrictions of the study population can confer advantages with respect to confounding control, cooperativeness, accuracy of data collection, and optimal distribution of exposure. These advantages may be crucial to obtain an estimate of association that is valid within the studied population, which is a prerequisite for any external inference to be made. Restrictions will impair generalizability only if the characteristics that distinguish the studied population could reasonably modify the effect of the exposure.[184]

The Swedish Mammography Cohort (SMC) and the Cohort of Swedish Men (COSM) are population-based cohort studies, to which all women and men in a certain age range and residential area were invited to participate. The participation rate was 74% in the first SMC recruitment in 1987–1990 and 70% for the 1997 questionnaire. For the COSM, 49% of the invited men participated. Despite non-complete participation, the cohorts have been demonstrated to be representative of the Swedish population of middle-aged and older people, with regard to distribution of age, BMI, and educational level.[166] Our results from **paper III** and **paper IV** can thus be considered to be directly generalizable to similar populations of middle-aged and older people in Sweden and other comparable countries. However, generalizability across geographical regions may be limited, as highlighted in **paper I**, because of differences in the characteristics of the fish consumption as well as potential genetic differences.

For **paper II**, the potential impact of a non-representative sample is important to consider, as it is inherent in the aim of a validation study to be able to generalize the results from the subcohort to the entire cohort. Those willing to participate in the clinical subcohort may be more interested and motivated, and our results of validity may therefore be a slight overestimation of the questionnaire performance in the cohort as a whole. Moreover, **paper II** was based on women only, and generalizability to men may be somewhat limited.

6.1.4 Meta-analysis

Meta-analysis is a method for combining results from published studies on the same association. It can be a valuable tool to gain statistical power and to condense rapidly growing amounts of literature and accumulated evidence. It can also be a tool for contrasting results and identify patterns and sources of heterogeneity, as was the case in **paper I**.

A meta-analysis naturally inherits the limitations of the included studies. Such limitations cannot be corrected and has to be considered when interpreting the summarized results. All studies included in **paper I** were of prospective design, and the strengths as well as potential limitations discussed above apply to them as well as to the original prospective cohort studies in this thesis. Additional sources of uncertainty are added by the combination of data from studies with different study protocols, and by necessary assumptions to allow combination.

A further specific limitation of meta-analyses is the issue of publication bias. This stems from a tendency of papers being submitted, published, and cited depending on the strength and direction of association. Studies reporting “desirable” results may thus be more likely to be included in a meta-analysis, which would introduce bias. A common technique to assess publication bias is the funnel plot, in which the precision is plotted against the effect size for each included study. In the absence of publication bias, the plot should be symmetrical. The symmetry is evaluated statistically by the Egger regression asymmetry test. In **paper I**, no publication bias was indicated by this test.

6.2 MAIN FINDINGS AND INTERPRETATIONS

6.2.1 Validity of questionnaire-based exposure estimates

Results from **paper II** indicate reasonable capability of the FFQ to rank participants according to long-chain omega-3 fatty acid intake and fish consumption. The validity correlations for long-chain omega-3 fatty acids are in the upper range of most previously reported correlations with adipose tissue content.[185-195] The strength of correlations however depends largely on the between-person variation in the population, which limits direct comparability between studies.[196] The observed correlations with long-chain omega-3 fatty acid intake estimates derived from questionnaire assessments up to fifteen years prior to adipose tissue sampling support their usefulness in studies with long-term follow-up.

By using an objective biomarker not based on self-reports we were able to evaluate the questionnaire performance against a measurement with independent sources of error. Adipose tissue provides the best biomarker reflection of intake of non-endogenously synthesized fatty acids over the preceding one to three years.[197, 198] Although dietary intake is the primary determinant of long-chain omega-3 fatty acids in the body, it should be noted that the adipose tissue content is expressed as percentage of total fatty acids and thus includes endogenously synthesized fatty acids in the denominator. The lower correlation between FFQ-based estimates and adipose tissue content among participants with higher body fat percentage may be a result of a dilution by excess body fat. The adipose tissue concentration among leaner women might therefore be a better reflection of actual dietary intake, and the higher correlation in this group a better reflection of the FFQ validity. It is also possible that high-fat foods were selectively underreported among obese women,[199] which may have led to an overestimation of relative long-chain omega-3 fatty acid intake in this group.

6.2.2 Fish consumption in relation to type 2 diabetes incidence

In **paper I**, we reported the first systematic review and meta-analysis of prospective studies on the risk of type 2 diabetes by fish consumption and dietary intake of long-chain omega-3 fatty acids. Results from this large analysis including 24,082 cases among 527,441 study participants revealed substantial heterogeneity across geographical regions. The summary risk estimates indicated no association in European studies, a direct association in US studies, and an inverse association in Asian/Australian studies. Several additional meta-analyses were published around the same time and reached similar conclusions.[91-96]

We noted that the number of studies conducted outside the US was limited. Since the publication of **paper I**, five new prospective cohort studies have been published, all based on European populations. Taking this new evidence into account in an updated meta-analysis, the **paper I** conclusion of no association in European studies still holds. No associations were observed in meta-analyses by types of finfish (fatty or lean). For shellfish, the results were

conflicting between regions with an inverse association in two Asian studies and a direct association in two European studies.

Among the evidence adding to the updated meta-analysis was the study in the COSM (**paper III**). In this large cohort of Swedish men, the results were largely in line with previous evidence from European populations, with no overall association between total fish consumption and risk of type 2 diabetes. In a second multivariable model additionally adjusting for dietary exposure to environmental contaminants present in fish, there was a nonsignificant inverse association. It is possible that regional differences in exposure to contaminants via fish in part can explain the inconsistent results on the association between fish consumption and diabetes risk. **Paper III** is to our knowledge the first study to report results on this relationship taking dietary exposure to polychlorinated biphenyls (PCBs) and methylmercury (MeHg) into account. However, hair mercury measured in a Finnish study did not modify the association of fish consumption, nor of serum or dietary long-chain omega-3 fatty acids, with risk of type 2 diabetes.[18] The statistical "removal" of the influence of PCBs and MeHg in **paper III** is naturally only theoretical. For the population to fully benefit from the overall positive aspects of fish consumption, this needs to be replaced by active efforts to reduce environmental levels, paralleled by support for making sensible fish consumption choices.

We further observed that fried fish consumption was associated with a higher risk of type 2 diabetes. Different consumption and cooking habits between populations could thus be another possible explanation for the heterogeneity among studies of fish and diabetes risk. Among previous studies, one reported no association with fried fish consumption as opposed to an inverse association with total fish.[9] Another study reported that adjustment for fried fish consumption attenuated a direct association with total fish.[10] Possible explanations for differences in association by frying include increased energy-density, changes in fatty acid composition,[200] or adverse effects by compounds formed during the frying process (such as advanced glycation end products and heterocyclic amines).[201, 202] It is also possible that frying is a marker for other behaviors that influence the risk of type 2 diabetes.

The inconsistent results for shellfish across regions warrant further investigation. Three additional studies presented results on shellfish consumption but did not report sufficient information to be included in the updated meta-analysis. The EPIC-Norfolk study supported a direct association in European populations with an odds ratio of 1.36 (95% CI 1.02–1.81) comparing at least one serving per week with less than one serving per week. Another European study reported a relative risk of 1.04 (95% CI 0.61–1.77) for consumers *vs.* non-consumers. In the only US study on shellfish, consumption of shrimp, scallops, and lobster at least one time per month was associated with a relative risk of 1.06 (95% CI 0.97–1.13). In **paper III**, we observed a direct association that was not markedly influenced by adjustment for dietary PCB and MeHg exposure. Consumption habits could be of importance also for the observed results on shellfish. For example, in Sweden, and some other countries, shellfish is often consumed with mayonnaise or other fatty condiments.

6.2.3 Fish consumption in relation to cardiovascular disease and mortality among people with type 2 diabetes

The findings from **paper IV** support that fish consumption is associated with a lower risk of MI but not with stroke among women and men with type 2 diabetes. The results for both total and CHD-related mortality were in the inverse direction but not statistically significant.

As covered in the background section of this thesis, the proposed cardiovascular benefits of fish consumption have largely been attributed to the long-chain omega-3 fatty acid content, but may also be related to a wider range of nutrients. The overall observational evidence from studies in the general population supports an inverse association of fish consumption with cardiovascular disease and mortality.[3-5, 157] As type 2 diabetes is characterized by metabolic abnormalities, dietary recommendations for this group can however not simply be based only on results from studies in populations without diabetes.

Our findings are largely in line with the limited evidence from studies in populations with diabetes. Only three studies have assessed fish consumption in relation to incident CHD or MI, of which two included very small numbers of cases and observed no clear associations.[21, 22] In the substantially larger Nurses' Health Study, there was a strong inverse association which was suggested even at very modest total fish consumption of one to three servings per month.[20] In our data, the lower risk of MI was also observed starting at the more moderate consumption levels. We could however not use a reference group with lower intake due to the limited number of participants reporting fish consumption of less than one serving per month.

Fish consumption in relation to total mortality among people with diabetes has been addressed in three previous studies. In the Nurses' Health Study, a similar strong inverse association as for CHD incidence was observed.[20] Another US study reported a modest nonsignificant inverse association,[158] and a smaller Greek study including only 80 deaths reported no association.[159] To our knowledge, there are no previous studies specifically on CHD-related mortality. **Paper IV** is also the first study to report on the risk of stroke by fish consumption in a population with diabetes at baseline. Our observation of no association among women and men with type 2 diabetes is in contrast to the overall evidence from observational studies in the general population, as summarized in a meta-analysis.[5] The reason for this discrepancy is unknown and further studies are needed to confirm or refute our findings.

For MI as well as for total and CHD-related mortality, we observed associations with fried fish consumption that were largely in line with the associations for total fish consumption. One of the previous studies of total mortality also assessed fried fish separately, and reported a similar nonsignificant inverse association as was observed for total fish consumption.[158] Our results on stroke were somewhat different for total and fried fish, which is more in line with some previous studies in the general population that have reported discrepancies in results for total and fried fish in relation to CHD, stroke, and other cardiovascular disease and mortality outcomes.[203-207]

7 CONCLUSIONS

Results from the papers included in this thesis showed that:

- The accumulated observational evidence on fish consumption and dietary intake of long-chain omega-3 fatty acids in relation to type 2 diabetes risk is largely heterogeneous across geographical regions, with a higher risk observed in studies conducted in the US, no association in European studies, and an inverse association in Asian/Australian studies.

- Food frequency questionnaire (FFQ)-based estimates of long-chain omega-3 fatty acid intake and fish consumption have reasonable validity and long-term stabilty, supporting their use in studies of diet–disease associations with long-term follow-up.

- In a Swedish population of middle-aged and older men, there was no overall association between total fish consumption and type 2 diabetes incidence. Our results indicated that dietary exposure to environmental contaminants such as polychlorinated biphenyls (PCBs) and methylmercury (MeHg) may influence the relationship. Fried fish and shellfish consumption were associated with higher risk of type 2 diabetes.

- Among Swedish middle-aged and older women and men with type 2 diabetes, fish consumption was associated with lower risk of myocardial infarction (MI), while there was no support for an association with stroke. The results for total and coronary heart disease (CHD)-related mortality were inconclusive with some suggestion of lower risk associated with moderate fish consumption.

Overall, the results presented in this thesis do not challenge the current public health recommendations on regular fish consumption, neither for the general population nor for the high risk group of type 2 diabetes patients. General advice may however be too imprecise, and support for making sensible choices with regard to preparation methods as well as fish species varying in contamination may be needed in order for the population to fully benefit from increased consumption.

8 FUTURE RESEARCH

The results from this thesis contribute to the accumulating knowledge about the associations between fish consumption and risk of incident type 2 diabetes as well as some of its cardiovascular complications. Further research is needed to deepen the understanding and address remaining knowledge gaps, as proposed below.

Food and nutrients are not consumed in isolation and thus need to be considered in the context of overall diet. Synergistic effects of dietary factors as well as interactions between diet and genes need to be addressed by future research. It would also be of interest to evaluate individual foods in comparison with their relevant alternatives using substitution analyses. For example, fish consumption should be evaluated as an alternative to other protein sources such as different types of meat. There is also a need to investigate more specific subtypes of foods, as well as preparation methods. With regard to exposure assessment, self-reported data on diet should ideally be complemented with objective biomarkers that reflect dietary intake. Repeated measurements are also desirable to account for changes in habits over time.

More detailed dietary assessments are warranted to better capture exposure to contaminants via fish consumption. Self-reported data should ideally include a larger variety of fish species as well as their origin, and should preferably be complemented with measurements of objective biomarkers. More studies to directly assess the role of dietary contaminant exposures in relation to risk of type 2 diabetes as well as cardiovascular disease outcomes and mortality are needed. Elucidating their roles and at what doses they may be influential is necessary to understand how to optimize the health benefits of consuming fish.

Most of the available evidence on dietary factors for diabetes prevention has been generated from studies in high-income countries. In view of the rapidly increasing burden of type 2 diabetes and its complications in low- and middle-income countries, there is a need for more studies in other regions of the world where susceptibility as well as food consumption habits may differ.

There is still very limited evidence from studies on dietary factors in relation to complications among people with diabetes, and thus gaps in the scientific basis for dietary recommendations in this group. This applies to the macrovascular complications studied in this thesis, but even more so for microvascular complications. Future studies are therefore warranted to evaluate the role of dietary factors in relation to complications of the eyes, kidneys, and nerves.

The recent years' improvement in coverage of the Swedish National Diabetes Register is reassuring for future epidemiological studies in Sweden, and will contribute to improved identification of prevalent and incident diabetes cases. More comprehensive reporting of

other variables will further enhance the possibilities of considering data on treatment, glycemic control, and other diabetes complications.

Finally, beyond the important objective to improve understanding of the etiological role of diet, effective dietary management strategies for patients with type 2 diabetes require evaluation of adherence to recommendations. There is also a need to integrate knowledge of diet–disease relations with consideration of environmental implications of food choices to promote healthy habits that are sustainable in a broader perspective.

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