Metabolic syndrome, gastrointestinal hormones and persistent organic pollutants in morbid obesity and effects of diet on persistent organic pollutants

PhD thesis

Sasha Dushanov

Oslo University hospital

Department of endocrinology, obesity and Preventive medicine

Section for Preventive cardiology

Table of contents

Acknowledgements

List of papers

Acknowledgements

This PhD thesis has been a long and exciting journey for me. That journey was not possible without people who supported me along the way, helped me, taught me and finally comforted me when things went wrong. Please note that during this journey I changed the spelling of my name from Sasa Dusanov to Sasha Dushanov.

With this I would like to thank first and foremost the best mentor in the world, Serena Tonstad. You have given me the opportunity to work on this project. You gave me the insight into the research world, helped and taught me during my research. You have had endless understanding and tolerance for all my challenges, and I can assure you, I had plenty of them during these 5 years. You have always been clear, quick with answers and very committed, even if you have been indisposed. I appreciate that and I thank you.

It is an honor for me to get to know people outside my own clinical world. My co-supervisor Jerome Ruzzin has helped me understand the underlying biological mechanisms that we clinicians often do not see or understand.

Thanks to Tor Ole Klemsdal for sincere, professional and intellectual support for this work.

Mette Svendsen and Eli Heggen, you have been an incredible resource for me with all your knowledge in your fields.

Thanks to Tine Sundfør who fortunately has been a notch ahead of me in the PhD process which has made my entire work much easier. I have learned a lot from you.

My sincere thanks to my co-supervisor Kåre Birkeland for expert guidance with the overall thesis.

Thanks also to our partner Hannu Kiviranta and his team from Finland for professional work on blood tests, and co-authorship.

Thank you so much to co-authors who have contributed to completing the articles, Lars Retterstøl, Hanne Gulseth, Kari Brandal, Panu Rantakokko, Airaksinen Riikka, Srdjan Djurovic and Teresia Wangensteen.

My PhD project would not be possible without support from Preventive cardiology section, Department of endocrinology, morbid obesity and preventive medicine, Oslo University Hospital, Extrastiftelsen via Norwegian Health Association and ‘’Norgesgruppen’’ with Aina Maria Lien.

Thanks to my best colleagues at Kløfta Medical Center: Espen Devold, Anne-Kjersti Staveteig, Cathrine Tølløfsrud, Ingebjørg Myre Hilstad and Jone Vistnes. You have given me confidence and so much freedom for this job that without it, the PhD program could not have happened.

In my opinion statistics is one of the most challenging fields in research. Lien My Deep has helped me a lot by the transfer of her knowledge.

Thanks to our two outstanding nurses, Ragnhild Kleve and Lise Bergengen. Without your help and knowledge of practical things in study execution the job would have been much more difficult.

Thanks to Tonje, Lisa and Anne-Britt for assistance with administrative questions.

Thanks to Synnøve Thon and Gabriele Foss for advising our study participants and data collection.

Lastly, I want to thank my family, my father Slavko and my mother Rajna who have always taught me to look up, be intellectual and seek knowledge no matter how long and distant it may be. Thanks to my younger brother Darko for being so honest.

Thanks to my two diamonds, Aleksej Filip and Nikolaj Filip for giving me strength even though there was no strength available. I apologize to you for every second you have been without me while I worked on the project.

And my dearest Jelena, you are my muse, my inspiration and my everything, I love you, thank you for believing in me.

Abbreviations

ANOVA	Analysis of variance
BMI	Body mass index
CHD	Coronary heart disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DXA	Dual X-ray absorptiometry
HbA_{1c}	Hemoglobin A_{1c}
α-HCH, β-HCH, γ-HCH	hexachlorocyclohexanes α, β and γ
PBDEs	Polybrominated diphenylethers
PeCB	Pentachlorobenzene
POPs	Persistent organic pollutants
PCBs	Polychlorinated biphenyls
p,p'DDE	Dichlorodiphenyldichloroethylene
p,p'DDT	Dichlorodiphenyltrichloroethane
RCT	Randomized controlled trial
WHO	World Health Organization

List of papers

Paper I

Associations of Circulating PYY_{3-36} Concentrations with Metabolic Syndrome in Extremely Obese Subjects

Dusanov S, Brandal K, Heggen E, Tonstad S.

Metab Syndr Relat Disord 2016; 14(8): 410-15.

Paper II

Characteristics of Metabolic Syndrome in Morbidly Obese Subjects

Dusanov S, Heggen E, Tonstad S.

Metab Syndr Relat Disord 2016; 14(10): 500-6.

Paper III

Associations between Persistent Organic Pollutants and Metabolic Syndrome in Morbidly Obese Individuals

Dusanov S, Ruzzin J, Kiviranta H, Klemsdal TO, Retterstøl L, Rantakokko P, Airaksinen R, Djurovic S, Tonstad S

Nutr Metab Cardiovasc Dis 2018; 28(7): 735-42.

Paper IV

Effect of fatty fish or nut consumption on concentrations of persistent organic pollutants in overweight or obese men and women: a randomized controlled clinical trial

Dusanov S, Svendsen M , Ruzzin J, Kiviranta H, Gulseth HL, Klemsdal TO, Tonstad S.

Nutr Metab Cardiovasc Dis 2020; 30(3):448-58.

1 General introduction

1.1 Obesity – epidemiology and pathogenesis

Obesity represents one of the most important and complex public health challenges medical science faces today. The World Health Organization (WHO) updated key facts on obesity and overweight in 2018 and reported that the prevalence of obesity has nearly tripled since 1975 (1). Among children younger than 5 years 41 million and among children aged 5-19 years 340 million were overweight or obese in the same year (2). Latest updated data from WHO show that almost 2 billion adults (39% of the global population) were overweight and 650 (13%) millions of these were obese in 2016 (1). Cardiometabolic disorders (metabolic syndrome, type 2 diabetes) are increasing as health burdens on the population, following the global increase in prevalence of obesity (3).

In Norway, data from 2017 showed that about 15-20% of all children were overweight or obese. These proportions have not changed much in the last decade (4). The HUNT-3 study showed that among Norwegian adults, about 54% of 40-49 year old men and 37% of women of the same age were overweight, while 24% of men and 21% of women were obese (5). Immigrants are noted to carry higher risks of metabolic disturbances related to obesity in Norway than non-immigrants (6).

Understanding the pathogenesis of obesity requires the integration of molecular, genetic, psychological, behavioral and environmental pathways and risk factors. The brain, in particular the hypothalamus, plays a key role in the homeostatic regulation of energy intake, metabolism and expenditure. Essentially there are 2 distinct and related processes that determine the pathogenesis of obesity. These include firstly, sustained positive energy balance (energy intake that is greater than energy expenditure) and secondly, resetting of body fat stores at an increased level (7). The set-point hypothesis of body fat regulation was proposed

in 1953 (8) and is supported by evidence from monogenetic mutations causing obesity, from clinical observations of how difficult it is for obese persons to maintain weight loss and from evidence related to the integration of signals from peripheral hormones and neurotransmitters in the hypothalamic appetite regulation center to maintain fat stores.. Much progress has been made in understanding the molecular basis of this regulation. However, the notion is also disputed because of the lack of strong biological signals to reduce weight back to normal when weight is gained (9). It does appear that generally there is a rather narrow range for individuals within which body fat stores are sensitive to environmental and lifestyle factors while the placement of the range may be related to genetic factors.

Heritability of body mass index (BMI) tends to settle at over 75% and is higher than for several other phenotypic traits (10). Genetic causes of obesity can be defined as monogenetic (single gene mutations, such as those related to the leptin pathway), syndromic (obesity associated with other phenotypes) and polygenetic (large numbers of small changes in genes that predispose to obesity) (11). In polygenetic obesity further research is needed on how to prevent individuals from reaching their highest potential for body fat stores given the difficulty of treatment to date.

Stages of life contribute in various ways to development of obesity. The perinatal milieu is a very early factor affecting the development of neural pathways and organs that contribute to the regulation of energy and metabolism, as well as glucose homeostasis (12). In childhood several contributors to the onset of obesity may appear. A prospective cohort study described adverse childhood experiences (parental divorce, familial alcohol/drug abuse, death of close relatives, serious illness, staying in a foster home, a parent with a mental illness, a move to another house/country, conflicts between parents) that are associated with higher risk of developing overweight in early adolescence, especially among children living in lower-income households (13) .

Furthermore, as is well known, unhealthy lifestyles in adulthood such as inadequate exercise, sedentary behaviors, and dietary patterns are among the most important causes of obesity (14). All these contributors may be affected by psychosocial conditions, socioeconomic patterns and chronic psychosocial stress and appear to be difficult to modify in the long term. Psychiatric disorders, in particular depression, play important roles in development of overweight and obesity, while evidence for anxiety is modest (15). Some medicines used to treat mental health disorders, such as antipsychotics and certain antidepressants may contribute to weight gain (16). Newer studies suggest novel contributors, including the subtypes of microbiota in the gastrointestinal tract may be associated with obesity and related metabolic disorders (17).

Hence, obesity is the result of a complex interaction between the individual's genetic disposition, factors related to social and cultural habits, and environmental factors. Among environmental factors a large number have been described including mechanized transport to work/school, inactivity and immobility, environmental pollutants, rapid eating, portion size and junk foods - both of which are usually supported by publicity in mass media, socio-cultural and ethnic factors, poverty and seasonal light/dark photoperiods (18). This thesis will focus mainly on pollutants and their impact on obesity and cardiometabolic risk regarding questions that remain open to further research (19), in particular in persons with high levels of obesity.

1.2. Overweight and obesity – definition

According to the WHO, overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. A person with a BMI of 30 or more is generally considered obese. A person with a BMI equal to or more than 25 but below 30 is considered overweight (20).

Cut-offs for overweight and obesity have been defined by several medical and public health organizations. There is consensus generally regarding the following definitions proposed by WHO as shown in Table 1.

Table 1. Classification of overweight and obesity

Overweight	BMI ≥ 25 kg/m^2
Obesity	BMI ≥ 30 kg/m^2
Morbid obesity	BMI ≥ 35 kg/m^2 with presence of 1 or more comorbidities related to obesity
Extreme or severe obesity	BMI ≥ 40 kg/m^2

The American Heart Association has suggested that persons with BMI higher than 40 kg/m^2 may be defined as extremely obese (heart.org). This degree of obesity carries marked health risks. A pooled analysis of 20 prospective studies found that extreme or severe obesity (Class III obesity) is associated with considerable shorter life expectancy, mainly due to heart disease and diabetes, as well as cancer, compared to individuals with normal weight (21). The same study showed that about 6% of US adult population is extremely obese, and that compared to a normal weight population, they had a risk of 6.5-13.7 years of life lost (21). Extreme obesity has increased relatively more than the increase in mean BMI in recent decades, although less rapidly in the last years than prior to 2005 (22), but has received less focus in scientific research than lesser degrees of obesity. This thesis has its focus particularly on morbid and extreme obesity in papers I-III.

1.3 Role of adipokines and gastrointestinal hormones

Regulation of body fat stores centrally responds to peripheral signals from adipose tissue, the gastrointestinal tract and other organs. Scientific perception of adipose tissue has evolved from it being an energy storage and somewhat inert tissue to a highly active endocrine organ. One of the most important findings in this field was the identification of leptin in 1994, which soon after led to the discovery of other adipokines including resistin, adiponectin, interleukin-6, visfatin, ghrelin, and many others (23). Adipokines are metabolic hormones, produced and secreted by the adipose tissue that determine and control processes related to obesity, such as glucose intolerance, dyslipidemia, blood pressure, endothelial dysfunction, and inflammation, and are associated with other risk components of the metabolic syndrome (24).

Leptin is an important regulator of metabolic homeostasis and inhibits appetite and food intake (25). It is produced in adipose tissue and regulates energy homeostasis, neuroendocrine function, metabolism, immune function and other systems through its effects on the central nervous systems and peripheral tissues (26). Leptin is associated to insulin resistance and its production correlates with the amount of body fat (27). While people with neuroendocrine abnormalities, lipodystrophy and monogenic leptin deficiency may respond to leptin administration, obese people are mostly resistant to leptin treatment (26).

The gastrointestinal tract is the largest endocrine organ in the body and secretes over 30 regulatory peptide hormones. Gastrointestinal hormones secreted from specialized cells in the gastrointestinal tract play an important role in gastric emptying and the feeling of hunger and satiety. The "gut-brain axis" is a term that refers to several gastrointestinal hormones including glucagon-like peptide 1, pancreatic polypeptide, ghrelin and peptide YY (PYY) (28). PYY_{1-36} is released by endocrine L-cells in the small bowel and colon and cleaved by the enzyme dipeptidyl peptidase-4 to the major circulating form that is more bioactive, designated as PYY_{3-36} (29). PYY_{3-36} acts as a major inhibitor of food intake due to its high affinity for the

presynaptic inhibitory Y2R neurons in the hypothalamic arcuate center and Y2R in afferent vagal fibers (29, 30). Fasting concentrations are influenced by adiposity, age, gender, and lifestyle while postprandial concentrations are stimulated by energy load and macronutrient composition as well as exercise (30, 31). Some studies have reported that obese individuals have lower basal fasting levels of PYY_{3-36} and have a smaller rise in postprandial levels (32). This hormone has been linked to systolic blood pressure in obese individuals (33), but its links to cardiovascular risk factors in persons who are morbidly obese have not been clarified.

1.4 Healthy and unhealthy obesity

Vulnerability to the metabolic consequences of obesity differs among individuals. In recent years researchers have contrasted healthy and unhealthy obesity, and obese individuals without metabolic abnormalities are categorized as metabolically healthy (34). These people might be protected from metabolic complications of obesity or at least have a lower risk of metabolic complications (35). Up to one-third of all obese people are considered metabolically healthy (36). This heterogeneity may be due to genetic, behavioral, and biological and environmental exposures (37). These differences may start in childhood (38). Possible mechanisms that can contribute to understanding this phenotype are poorly known. Lesser degrees of insulin resistance and subclinical inflammation might play a differentiating role between these individuals and others with unhealthy obesity (36). Meta-analyses have reported that metabolically healthy obese individuals also carry high risks of cardiovascular events in the long-term compared to healthy lean individuals, but they were not associated with higher risk of premature all-cause mortality (39). However, the Tromsø study conducted in Norwegians showed that increase in BMI increased the risk of myocardial infarction, regardless of physical activity level (40). Metabolically unhealthy obese individuals often develop diseases that can reduce life quality and life expectancy and increase health-care costs (37). Unhealthy obesity is often defined by the concept of metabolic syndrome.

1.5 Metabolic syndrome

Metabolic syndrome is a clustering of metabolic and cardiovascular risk factors. It results from the interaction between environmental and genetic factors. It is defined clinically by the presence of cardiometabolic risk factors, namely abdominal obesity, low HDL-cholesterol, high fasting triglycerides and glucose, and high blood pressure (41). The WHO defined metabolic syndrome first in 1999, but it has since been redefined by several organizations across the world.

The definition of metabolic syndrome used in this thesis is the harmonized one (41). It unifies criteria from the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute and uses the following cut-offs: waist circumference ≥102 cm for men and ≥88 cm for women; systolic blood pressure ≥130 mmHg and/or diastolic ≥85 mmHg, or drug treatment for hypertension; triglycerides ≥1.7 mmol/L; HDL-cholesterol ≤1.0 for men or ≤1.3 for women; and fasting glucose ≥ 5.6 mmol/L (41).

Metabolic syndrome is usually without symptoms, but waist circumference is a visible sign (42). Waist circumference is the most challenging component in regard to defining cut offs due to different genetic and biological factors among people in the world (41). The WHO has identified 2 levels of abdominal obesity in Europids depending on risk for metabolic complications. Increased risk occurs at waist circumferences of ≥94 cm in men and ≥80 cm in women, but risk is substantially higher at ≥102 cm in men and ≥88 cm in women. These higher levels are generally in use to define abdominal obesity in the United States, Canada, and Europe (43).

Individuals with three or more of the criteria in any combination components would qualify as having metabolic syndrome (41). Each of metabolic syndrome components singly increases the risk of cerebrovascular stroke, coronary heart disease (CHD) and diabetes but

whether the combination of factors is additive or multiplicative has been widely discussed. Most evidence points to additive effects and systolic blood pressure may be the most important component in terms of risk (44).

Prevalence of metabolic syndrome in US population is about 30% but prevalence may vary considering different criteria used in defining metabolic syndrome (45). Several meta-analyses suggested that detection, prevention, and treatment of metabolic syndrome is an important strategy in prevention of cardiometabolic diseases (46, 47). As an example the Scandinavian Botnia study showed that the risk of coronary artery disease and stroke increased threefold in patients with metabolic syndrome in the course of 6.9 years follow up (48). The Finnish Kuopio Ischemic Heart Disease Risk Factor Study, with approximately 11.4 years of follow up, showed associations between metabolic syndrome and deaths of all types of cardiovascular disease (CVD) and 2.9-4.2 times higher risks of CHD and 2.6-3.0 times higher risks of all CVD in men with metabolic syndrome (49). However different criteria used in defining metabolic syndrome may overestimate the risk of complications (50). The data from a meta-analysis of cohorts showed that individuals with metabolic syndrome were at 1.6-fold higher risk of having stroke than their counterparts without metabolic syndrome, while longitudinal population based study showed that metabolic syndrome and its main components can be potent predictors for long-term ischemic stroke (51, 52). Meta-analysis suggested that metabolic syndrome may be a particularly important risk factor for stroke in women (53). Overall, this evidence supports the notion that obese persons may have a particularly increased risk of CVD if they also carry the burden of metabolic syndrome.

Metabolic syndrome can also be a useful tool in clinical practice for predicting type 2 diabetes mellitus, showed a review from 2017 (54). Data from the well-known Framingham Heart Study Offspring Study supports this (55). Relative risk of diabetes development in

patients with metabolic syndrome was ranged from 3.53 to 5.17 in a meta-analysis of 16 multiethnic cohort studies (56).

1.6 Treatment of obesity, in brief

The degree of obesity does not necessarily correlate with the presence of metabolic syndrome (57), but reducing weight with as little as 5% can improve all metabolic syndrome components, and lowers risk of CVD and type 2 diabetes regardless of the degree of obesity (58). Implementing such weight loss by active education and effective dietary intervention may help to prevent diabetes and its complications including severe late complications (59).

It is well-known that energy restriction is necessary for weight reduction. Adherence to dietary programs is usually a challenge for most individuals struggling with obesity. A wide range of ways to achieve energy restriction have appeared with the notion that some may help more than others. These include low-fat diets, very-low-fat diets, moderate-fat diets, high-protein diets, low-carbohydrate diets, low-glycemic index diets, intermittent fasting and others. While these diets may differ in their effects on cardiometabolic risk markers, most result in improvements in health parameters when they are followed (60).

Physical activity has traditionally been considered as essential in weight reduction and it needs to be a part of every clinical intervention of obesity or overweight (61). Physical activity or exercise training alone is likely to promote moderate weight loss of not more than a few kilograms (62). However, despite this, physical activity is highly relevant for preventing CVD (63). All types of physical activity should be encouraged and need to be a part of lifestyle changes (64). Furthermore, a recent trial showed that physical activity can cause significant reductions of visceral adipose tissue, independent of weight loss caused by different diets (65).

1.7 Dietary prevention of CVD

Dietary patterns are important and well documented in prevention of CVD regardless of whether weight loss is achieved (66). Meta-analysis of cohort prospective studies highlight whole grains, dietary fiber, fruits and vegetables, nuts and seeds as foods that may reduce risk of CVD (67).

One of the diets that has long been linked to prevention of obesity and its complications is the concept of Mediterranean diet (68). The Mediterranean diet as consumed traditionally consists of bread, legumes, vegetables and fruits, nuts, olive oil, and limited red meats, meat products, butter, hard margarine and sugar as well as moderate alcohol intake (69).

A review that analyzed observational studies, randomized controlled trials (RCTs), cohorts and meta-analyses (conducted from 2014-2018) including totally 45 studies concluded that there is a large and consistent data of evidence that support the benefits of the ingredients of the Mediterranean diet on cardiovascular health (70). PREDIMED ((Prevención con Dieta Mediterránea (Prevention with Mediterranean Diet)) was a Spanish multicentric study, from 2013 (republished in 2018) which showed that individuals with high cardiovascular risk, had lower incidence of major cardiovascular events if they followed a Mediterranean diet supplemented with extra-virgin olive oil or nuts than among those assigned to a reduced-fat diet (Hazard ratio 0.69 [95% confidence interval 0.53-0.91] and 0.72 [95% confidence interval, 0.54-0.95], respectively) (66). Acceptance and feasibility of the Mediterranean diet is limited in non-Mediterranean countries and in certain groups (71) and requires modification according to local culture and norms. In Norway, official dietary recommendations advise that the population should eat lots of vegetables, fruit, berries, whole

grains, legumes, nuts and fish and that processed and red meats, salt and sugar should be limited (72).

As indicated in these recommendations, ingestion of fish, especially fatty fish has been considered important in prevention of CVD. Fatty fish is rich in the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which may have protective roles due to anti-inflammatory effects, inhibition of platelet aggregation, triglyceride lowering effects, improving endothelial function and stabilizing atherosclerotic plaque (73, 74). Earlier studies, such as a RCT from 1989, as well as a review from 2002, showed that eating fish may reduce the incidence of CVD and also reduce mortality in men recovering form myocardial infarction (75, 76). Recently, a comprehensive literature search found that fish intake was associated with a lower risk of metabolic syndrome in prospective cohort studies but not in cross-sectional ones (77). A threshold of 400 g/week of fish was noted above which the risk of metabolic syndrome decreased significantly.

Similar reductions in CVD risk were not supported in some randomized controlled clinical trials (78). A meta-analysis published in 2012 showed that there was not sufficient evidence that omega-3 fatty acids had secondary protective effect in patients with CVD, and that some studies that showed protective results did not use placebo (79). The U.S. Departments of Health & Human Services suggests possible explanations for conflicting findings including the observation that people nowadays already ingest high amounts of omega-3 fatty acids, compared to earlier, and perhaps that widespread use of cholesterol lowering medication (statins) do not leave room for additional effect of fatty acids (80).

Differences between studies could be explained by the dosage of omega-3 that is used in studies. Omega-3 fatty acids supplementation given in high dosages (more than 1 g/day) may reduce risk of death in diabetic patients (81). Data from a meta-analysis from 2019

supported the thesis that ingestion of omega-3 fatty acids lowers risks of coronary artery disease and all cardiovascular deaths and suggested that the effect may be linearly related to the omega-3 dose (82). A multicenter, randomized, double-blind, placebo-controlled trial involving patients with established CVD or with diabetes and other risk factors called "REDUCE-IT" showed that ingestion of 4 g EPA daily was associated with significantly lower risk of ischemic events in population with elevated triglycerides on top of use of statins (83). Furthermore, ingestion of fish and long-chain omega-3 fatty acids reduce mortality from major causes (84). Usual recommendations of fatty fish intake (1-2 servings per week) do not usually reach omega-3 levels of 1 g/d, which might be necessary for primary prevention (81).

Another dietary component that has been studied in detail regarding CVD prevention is nuts. Nuts are rich in protein, fiber, unsaturated fatty acids and phytochemicals and are easy to incorporate into the diet. Evidence links nuts to reduced rates of CVD (85) and overweight (86). Based on three prospective studies, high nut consumption was inversely correlated with total CVD and CHD (87). A meta-analysis and systematic review from 2018 found that eating nuts was associated with lower all-cause mortality, mortality caused by CVD and CHD, but not associated with a lower risk of type 2 diabetes (88).

1.8 Persistent organic pollutants (POPs)

Dietary intakes differ not only regarding macronutrient and micronutrient compositions, but also in regard to their content of environmental pollutants. POPs are lipophilic chemicals that bioaccumulate in living organisms for decades. In the main, POPs are stored in adipose tissue, but are also measurable in serum and may vary according to age and gender (89). Factors affecting the homeostasis between POPs stored in adipose tissue and circulating blood concentrations are not clear. These associations may differ according to the compound and previous exposure (90). Some authors showed that common predictors for

elevated levels of POPs were birth year, breastfeeding and the weight-related variables, while dietary variables were of varying importance (91).

According to WHO, POPs are chemicals of global concern worldwide, due to their negative effects on humans, having specific characteristics such as long-range transport, persistence, bio-magnification (concentration in the tissues of organisms at successively higher levels in a food chain) and accumulation in the nature, and high contaminating capacity of the food, water and air, as well as of products that we use to improve their quality (92).

POPs may be grouped in several categories, mainly based on chemical structure or use. Usual categorization is: organochlorine pesticides including dichlorodiphenyltrichloroethane (p,p'DDT), dichlorodiphenyldichloroethylene (p,p'DDE), α -hexachlorocyclohexane (α-HCH), β- hexachlorocyclohexane (β -HCH), γ-hexachlorocyclohexane (γ-HCH), pentachlorobenzene (PeCB), hexachlorobenzene, *trans*-Nonachlor, and oxychlordane, polychlorinated biphenyls (PCBs), including dioxin-like (118, 156) and non-dioxin-like (74, 99, 138, 153, 170, 180, 183, 187) and polybrominated diphenylethers (PBDEs) 47, 99 and 153.

The negative effects of POPs on human health have been discussed for decades. More recently, focus has been on cardiometabolic disorders and diseases. In a recent cohort study individuals with higher levels of PCBs had higher risk of death, primarily from CVDs (93). Furthermore, in the epidemiological survey NHANES 1999-2011 exposure to some organochlorine pesticides was associated with higher all-cause mortality and with higher non-cancer, non-heart/cerebrovascular disease mortality in U.S. adults older than 60 years (94).

POPs appear to disrupt endocrine regulation of metabolism, possibly leading to weight gain and obesity (95). Both higher and lower POP levels have been reported in obese versus lean individuals. These associations may differ according to the compound and previous

exposure (90). Because studies are mostly cross-sectional or obtained data on weight change retrospectively (96), causation between POP levels and obesity remains speculative. Weight loss reduces adipose tissue storage increasing concentrations in blood in the short term, though drastic weight loss decreases the total body burden (97). Based on data from NHANES it has been suggested that obese people may safely store POPs in adipocytes, and that even though weight loss may have positive effects on health in individuals with low POPs concentrations, weight loss may contribute to health risks in those with high concentrations (98).

POPs contribute to increased risk of metabolic syndrome (99) and CVD as suggested in the PREDIMED-CANARIAS cohort study (100) and in a number of other studies (93, 101). The metabolically healthy, but obese phenotype, has been associated with lower plasma levels of POPs than the metabolically unhealthy obese phenotype (102). A meta-analysis including 72 epidemiological studies found sufficient evidence for a positive association of some POPs and type 2 diabetes, despite studies' heterogeneity (103). In individuals without type 2 diabetes exposure to various POPs predicted obesity, dyslipidemia and insulin resistance during 2 decades of follow-up (104). Several observational cross-sectional and prospective studies have suggested evidence for DDT/DDE compounds and even more for PCBs in increasing risks of developing type 2 diabetes. The Nurses' Health Study reported an association between plasma HCB concentration and incident type 2 diabetes (105). In the meta-analysis both HCB and total PCBs were associated with diabetes incidence after multivariate control, with odds ratios at or just below 2 (46).

1.9 POPs and diet

Populations are mainly exposed to POPs through the consumption of fat-rich foods of animal origin. Modifying dietary patterns may be useful to decrease POPs burden and the risk

of CVD (100). A study from Spain showed that the most consistent association between foods and concentrations of POPs was with fish followed by dairy and meat, while vegetables, fruits and cereals were rarely related to POPs levels (19). The British Food Standards Agency underlines that animal products may be the most important sources of POPs, that can occur through high exposure over a long time (106). Based on animal studies some authors suggest that accumulation of POPs is affected by macronutrient intake, and not the total intake of POPs. This finding may suggest the importance of controlling macronutrients in dietary studies of POPs (107, 108).

Earlier research found significantly higher burdens of POPs in farmed than wild salmon and farmed salmon from Europe were significantly more contaminated than farmed salmon from South and North America (109). In the recent years aquaculture feed has been increasingly based on plant oils, instead of feed of animal origin (110). A Norwegian study suggested that farmed salmon may have lower levels of POPs compared to wild salmon (111).

1.10 Summary of the introduction and basis for the thesis

Medical understanding regarding obesity, metabolic syndrome, and their contributors has improved, but further studies are necessary to understand the underlying pathways in development of these diseases and their association with CVD. Presence of POPs in the diet complicates these mechanisms further. In this research we focused on overweight, morbidly and extremely obese people with cardiometabolic risk. We have described metabolic syndrome in morbidly obese individuals, role of gastrointestinal hormones in extreme obesity, concentrations of selected POPs in people with morbid obesity, and we conducted a trial to understand the role of diet in the development of POPs in overweight and obese individuals using a high level of evidence, namely the randomized controlled design.

2 Aims of the thesis

The overall aim of this thesis was to examine the role of POPs in influencing metabolic disturbances in subjects with obesity, and whether a controlled diet with farmed salmon or nuts would result in significant changes in metabolic status and POPs concentration after 6 months intervention. A further aim was to describe the relation between specific gastrointestinal hormones and POPs with metabolic disturbances in morbid obesity. In paper I we explored the association of the gastrointestinal hormone PYY_{3-36} in extreme obesity in relation to metabolic syndrome. In paper II we studied cardiometabolic risks in morbidly obese men and women with the goal of better understanding of the expression of metabolic syndrome in this population. The main aim in the paper III was to examine levels of POPs and their relation to metabolic syndrome in morbidly obese individuals. Paper IV shows the effect of consuming foods rich in POPs cardiometabolic risk factors in overweight and obese individuals.

3 Hypotheses

1. Does the gastrointestinal hormone PYY_{3-36} correlate with degree of obesity in extremely obese people?
2. Do metabolic syndrome components and CVD risk factors correlate to BMI and fat distribution in morbidly obese men and women?
3. Do high concentrations of POPs correlate with expression of metabolic syndrome in morbidly obese people?
4. Does a high consumption of fatty fish over 6 months increase levels of POPs in overweight and obese men and women compared to a control group avoiding fatty fish and nuts?
5. Does consumption of fatty fish change markers of cardiometabolic risk in overweight and obese men and women in a period of 6 months?
6. Does a high consumption of nuts over 6 months change markers of cardiometabolic risks in overweight and obese men and women compared to a control group avoiding nuts and fatty fish?

4 Subjects and methods

Participants in papers I-III were consecutive adult patients aged 18 – 78 years referred to the Preventive Cardiology Clinic at Oslo University Hospital, Oslo, Norway between April 2005 and December 2010. Referrals were from primary care physicians or specialists for obesity treatment and cardiovascular risk factor reduction as needed. All had a BMI ≥ 30 kg/m^2 and are designated as a clinic-based obesity population in this thesis. They were asked to provide demographic data, anthropometric parameters, give blood samples for laboratory analyses, and take part in screening for the melanocortin-4 receptor gene mutations to assess the frequency of these mutations in a Norwegian population if their BMI was ≥ 35 kg/m^2 (112). They also consented to storage of blood samples in a biobank for future analyses. Only patients that gave written informed consent were included. The participation rate was >95%. All participants underwent a physical examination and a structured interview by a physician. They responded to 2 self-administered questionnaires regarding their current health, concomitant diseases, medical treatment, and personal and family history of obesity (112). As shown in figures 1a and 1b, subgroups of this clinic-based obesity population were included in papers I-III depending on the purpose of the study and availability of blood test results. A total of 64 participants (47 women and 17 men) with BMI between 30 and 34.9 kg/m^2 were excluded from all the papers due to the aim to focus on morbid obesity. Exclusions due to insufficient data to establish criteria for metabolic syndrome in 33 participants (19 women and 14 men) in paper I and 53 participants (31 women and 22 men) in papers II and III). Missing PYY values led to the exclusion of 172 participants (120 women and 52 men) from paper I. Diagnosis of diabetes in 209 participants (117 women and 92 men) led to exclusion in papers II and III. For paper III, 116 participants (74 women and 42 men) were missing samples for analysis of POPs and were excluded.

Figure 1a. Participant flow from clinic-based obesity population to Papers I-III

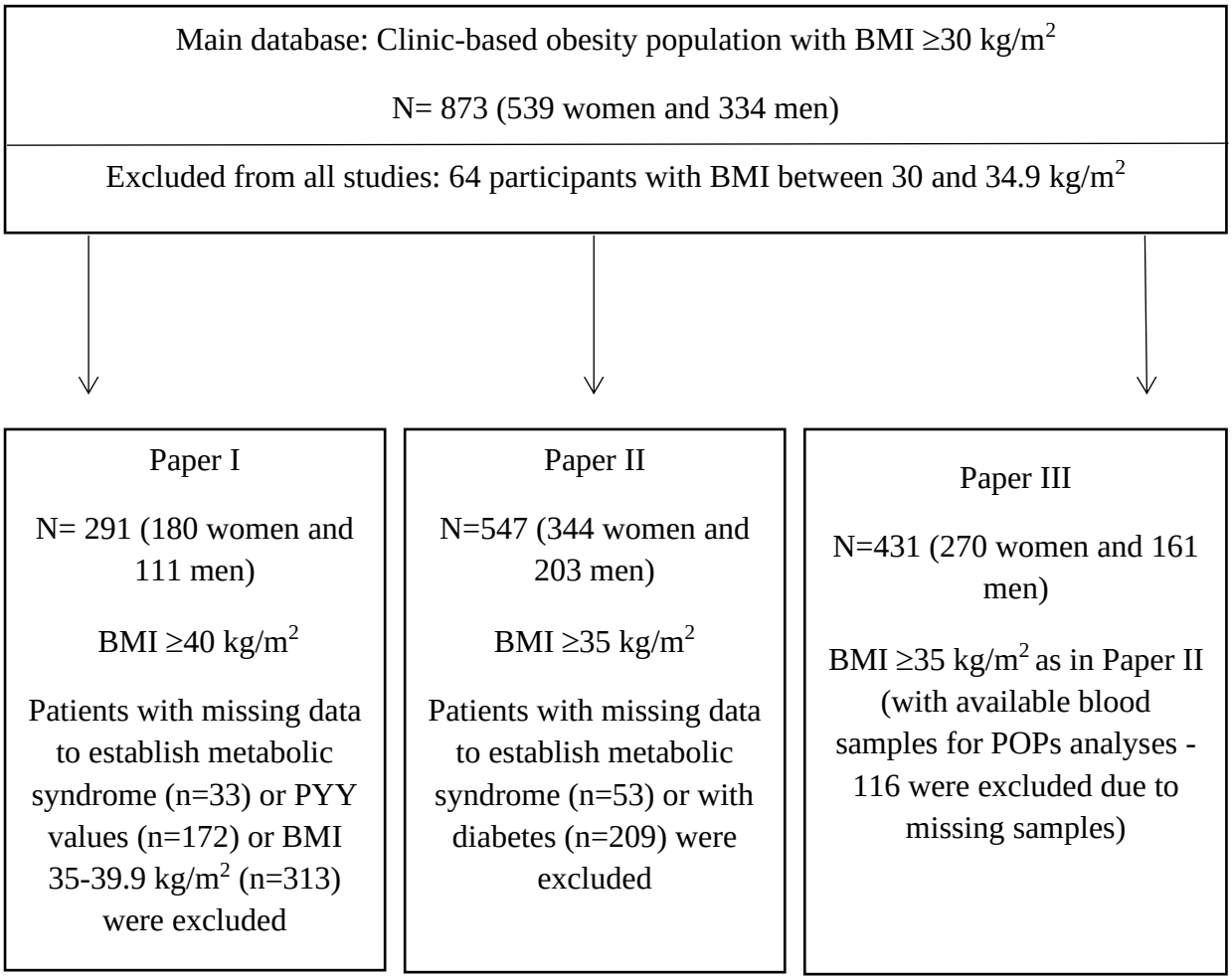
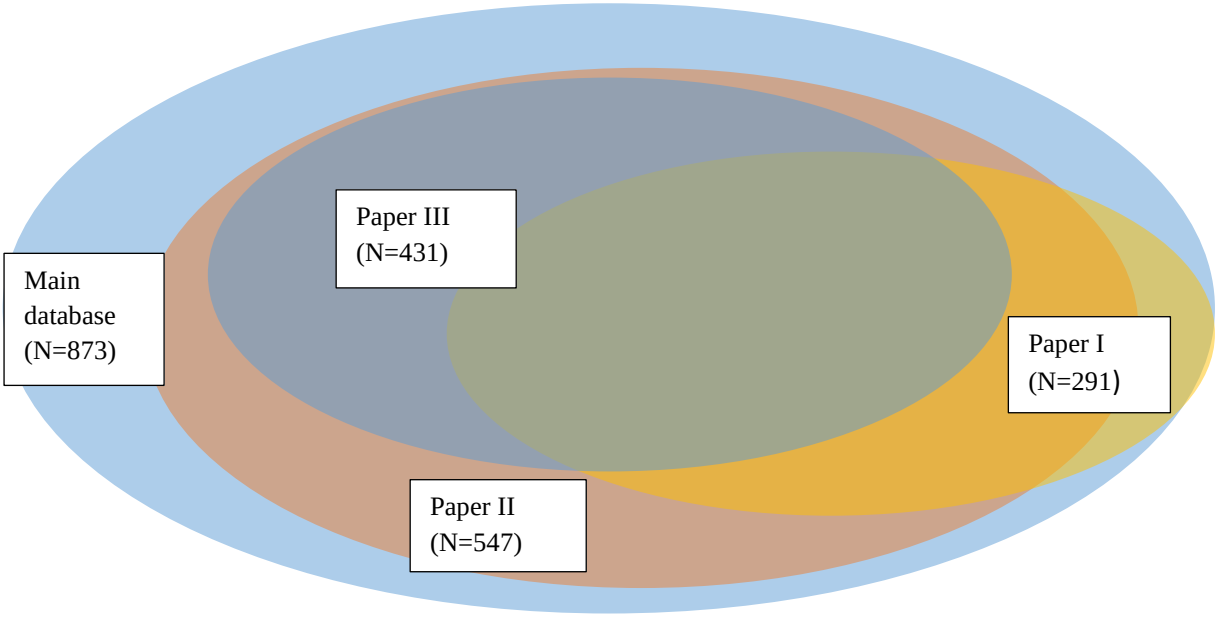


Figure 1b Participant distribution from clinic-based obesity population (Papers I-III)



For the anthropometric parameters, waist circumference was measured at the midpoint between the inferior costal margin and the highest point of the iliac crest, using a constant tension body tape. Height was measured using a stadiometer and recorded to the nearest centimeter. Weight was measured using a calibrated mobile electronic scale (Seca 720; medical Scales and Measuring Systems) and recorded to the nearest 1.0 kg. BMI was calculated using Quetelet's formula: weight in kilograms was divided by the square of height in meters.

Blood pressure was measured with an automatic blood pressure monitor 852000 Series Vital Signs Monitor; Welch Ally). Participants were seated and having rested for 5 minutes prior to using appropriate cuff size. Body total and regional fat percentages were analyzed by using dual X-ray absorptiometry (DXA) (Lunar DPX-L, Lunar) in 206 individuals. Only individuals with body weights below 140 kg could be examined by DXA. Others did not undergo the procedure for practical reasons.

Laboratory analyses included standard tests as shown in box 1.

Box 1. Standard laboratory tests obtained of all participants in Papers I-III

Metabolic syndrome and glucose-regulation parameters: Triglycerides, HDL-cholesterol, glucose, hemoglobinA_{1c}
Other lipids: Total cholesterol, LDL-cholesterol, apolipoprotein B, lipoprotein (a)
Inflammatory parameters: Ferritin, CRP, uric acid
Parameters of hepatic dysfunction: Alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase
Hematology analyses: Hemoglobin, white blood cell count
Electrolytes: Sodium, potassium
Kidney function: Creatinine
Thyroid: Free thyroxine, thyroid-stimulating hormone (TSH)

Paper I

We performed a cross-sectional study of a total of 291 (180 women and 111 men) aged 18-78 years with BMI ≥40 kg/m^2 (extreme obesity) in paper I. The range of BMI was between 40 and 74 kg/m^2. They were stratified by number of metabolic syndrome components (from 1 to 5) as follows: elevated waist circumference ≥102 cm for men and ≥88 cm for women, systolic blood pressure ≥130 mmHg and/or diastolic ≥85 mmHg, or/and use of medication for hypertension, triglycerides ≥1.7 mmol/L, HDL-cholesterol ≤ 1.0 mmol/L for men or ≤ 1.3 mmol/L for women, and fasting glucose ≥ 5.6 mmol/L. Subjects with fasting glucose ≥7.0 mmol/L or hemoglobin A_{1c} (HbA1c) ≥6.5%, or who had known type 2 diabetes, or used antidiabetic drugs were grouped together as the type 2 diabetes subgroup (22% of the sample). Of the total, 23% were cigarette smokers, use of blood pressure lowering medication was reported in 31.3%; among those without diabetes, 26.1% used anti-diabetic medication versus 49.2% of those with diabetes. Statin use was recorded in 8.9% of the entire sample; in 5.3% of those without diabetes and in 21.5% of those with diabetes. Parameters specific to the study were PYY_{3-36}, insulin and leptin, which were measured in addition to the standard blood tests. The Homeostasis Model Assessment Insulin Resistance index (HOMA-IR) index was calculated to estimate insulin resistance based on the Matthews formula: HOMA-IR = (Fasting plasma insulin × fasting plasma glucose) / 22.5 (113).

Paper II

In the second cross-sectional study a total of 547 participants (344 women and 203 men) with BMI ≥35 kg/m^2 and comorbidity (including hypertension, sleep apnea, dyspnea, polycystic ovarian syndrome, asthma, hypercholesterolemia, gout, musculoskeletal symptoms, gall bladder disease, esophageal reflux, pulmonary embolism or deep vein thrombosis, intermittent claudication, angina pectoris, depression, or eating disorder) or BMI

≥40 kg/m^2 regardless of comorbidities were included. The range of BMI was between 35 and 74 kg/m^2. Participants were stratified by number of metabolic syndrome components, using the same criteria as described for paper I. There were 209 participants with diabetes (as defined above in paper I) that were excluded. Of the total, 26% were smokers. 2/3 met criteria of metabolic syndrome. Standard blood test analyses were done. DXA analysis was performed in 206 participants. The paper's abstract erroneously indicates that total n was 549 participants.

Paper III

A total of 431 participants from paper II with adequate blood samples (116 participants did not have available blood samples) in the biobank for analysis of POPs were included in paper III. This cross-sectional study was conducted among 161 men and 270 women with BMI ≥35 kg/m^2 and comorbidity (see above), or BMI ≥40 kg/m^2. Of the total, 26% were smokers. The mean BMI was 42 kg/m^2, and almost 3/4 of men and 2/3 of women met metabolic syndrome criteria. Of the total 15% of men and 6% of women used statins. Insulin concentrations were measured in addition to standard blood tests and the HOMA-IR index was calculated. Circulating concentrations of 15 POPs (5 organochlorinated compounds, 2 dioxin-like PCBs and 8 non-dioxin-like PCBs) were measured and stratified by number of metabolic syndrome components.

Paper IV

This study was an RCT that included 131 participants (56 men and 75 women), of which 120 completed the study. Participants were aged 35-70 years with BMI between 25-38 kg/m^2. Participants were recruited through advertisement in newspapers, the Face-book page of Oslo University Hospital as well as from patient referrals to the Section of Preventive

Cardiology, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway. The first participant was included in January 2016 and the last 6-month follow-up was in October 2017. All participants signed written inform consent before starting any study procedures.

Inclusion criteria for the participation included 1 component of metabolic syndrome in addition to waist circumference (≥102 cm for men or ≥88 cm for women), i.e. blood pressure ≥130/85 mmHg or use of antihypertensive medication, fasting glucose ≥5.6 mmol/l, HDL cholesterol ≤1.3 for women or ≤1.0 for men, or triglycerides ≥1.7 mmol/l. Exclusion criteria were cigarette smoking, diabetes if treated with antidiabetic medication, allergy to or dislike of fish or nuts, chronic disease including cancer, gastrointestinal disease or CVD, morbid obesity (BMI of ≥38 kg/m^2 with obesity-related health conditions or ≥40 kg/m^2 alone) and weight fluctuations (+/- 5 kg in the last 6 months), eating disorder, history of bariatric surgery, use of anti-obesity drugs or other drugs affecting body weight.

The participants were randomized to high intakes of fatty fish (mostly farmed salmon, ~630 g/week; n=45), high intakes of nuts (~200 g/week; n=42) or a control group following their usual diet but restricting fatty fish and nuts for 6 months (n=44).

Body weight was measured using the same calibrated digital scale (Seca 877, Seca GMBH & co, Germany) to the nearest 0.1 kg. Height was measured using a stadiometer and recorded to the nearest centimeter. Waist circumference was measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Hip circumference was measured at the widest portion of the buttocks. Blood pressure was measured using the Omron 705IT (Omron HEALTHCARE, Kyoto, Japan) after the participant rested quietly in a sitting position for at least 5 min alone in a quiet room. The

mean of 3 measurements spaced 1 min apart was calculated at screening, randomization, and 3 month and 6-month visits.

Blood tests included analyses of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glucose, CRP and apolipoprotein B. HbA_{1C}, C-peptide and insulin were measured. HOMA-IR index was calculated and the hyperinsulinemic-euglycemic clamp was performed in a small subset (n=10 in fatty fish group, n=10 in control group). Concentrations of 15 POPs as mentioned above were measured. All blood tests were performed at baseline (randomization) and at the end of the study.

5 Laboratory analyses including POPs

In papers I-III the following methods have been used: Participants were instructed to fast overnight for at least 10 hours, before providing blood samples between 8:00 and 11:00 a.m. Analyses of blood samples were performed at Oslo University Hospital (Clinical Chemistry Laboratory at Ullevål or the Hormone Laboratory at Aker). Total cholesterol, HDL-cholesterol, triglycerides, glucose, alanine aminotransferase (ALT), uric acid, creatinine and high-sensitivity C-reactive protein (CRP) concentrations were measured on an automated analyzer Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). HbA1c was measured automatically with HbA1c Calibrator Set using TSKgel Variant HSi with stable s-A1c (Tosoh Automated Glycohemoglobin Analyzer HLC-723G7, Tosoh Corporation, Japan).

LDL-cholesterol was calculated using Friedewalds formula. Apolipoprotein B and lipoprotein (a) were determined with an immunoturbidimetric assay on an automated analyzer (Cobas Tinaquant 917, Roche/Hitachi, Roche Diagnostics, Mannheim, Germany). White blood cell count was analyzed using Sysmex XE 2100 (Sysmex, Mississauga, Ontario, Canada). Serum ferritin was determined by an ADIVA Centaur analysis (ADIVA Centaur,

Siemens Healthcare Diagnostics Inc., Tarrytown, USA). PYY_{3-36} and insulin assays were carried out between November 2010 and May 2011 on frozen samples. Serum PYY_{3-36} was measured using a radioimmunoassay kit which utilizes an antibody that only recognizes the $_{3-36}$ form of human PYY (Millipore, USA). The intra and interassay variations of coefficients were less than 15%, and the recovery was 85-129% by the linear range of the assay. The detection limit of the assay was 14 pg/mL (100µg sample size). The assay had a specificity of 100% for human serum $PYY_{3-36.}$ Insulin was determined by non-competitive immunofluorometric assay, using an AutoDelfia 1235 Automatic Immunoassay System (H1855-21291) (Perkin Elmer Inc.).

Serum leptin was measured on frozen samples using a human serum leptin radioimmunoassay (Luminex, Millipore, USA). The intra- and interassay variations of coefficients were <10%, and the recovery was 103-105% by the linear range of assay. The detection limit of the assay was 0.5ng/L (100µL sample size). The assay had a specificity of 100% for human serum leptin.

POPs were measured in 200 µl of serum at the National Institute for Health and Welfare, Kuopio, Finland. The following POPs were measured: PBDEs 47, 99 and 153; PCBs including dioxin-like (118, 156) and non-dioxin-like (74, 99, 138, 153, 170, 180, 183, 187) and organochlorine pesticides including p,p'DDT, p,p'DDE, α-HCH, β-HCH, γ-HCH, PeCB, hexachlorobenzene, *trans*-Nonachlor, and oxychlordane. The detection rate for PeCB, α-HCH, γ-HCH, oxychlordane and all PBDEs was <75%, and thus, these analytes were excluded from further statistical analyses.

A full description of the analytical method has been published previously (114). Limits of quantification for POPs were 5–40 pg/ml. In each batch of samples, a control serum sample from the National Institute of Standard and Technology, Standard Reference Material

(SRM) 1958 for POPs was included (n=13). An in-house produced low-level control sample was prepared by 1 to 9 dilution of SRM 1958 with newborn calf serum (NBCS) and also included in each batch of samples (n=13). Average concentrations for POPs from SRM 1958 were 87-111% of the certified/indicative concentrations. Coefficients of variation (CVs) were 1.4-4.4% for SRM 1958 and 1.8-8.9% for diluted SRM 1958. The Environmental Health Unit participates to AMAP inter laboratory comparison (Ring Test for POPs in human serum, National Institute of Public Health, Quebec, Canada) where 16 of the target POPs from three serum samples are reported for each round. During the last 2 years results from all samples for all POPs have been acceptable ($|z|<2$). Accuracy of the results for individual compounds from individual samples varied from 77-121 % of the assigned values.

Other blood tests in paper IV were performed as follows: Blood samples were obtained following a minimum of a 10-hour fast. Analyses of blood samples were performed at Oslo University Hospital Clinical Chemistry Laboratory/Ullevål. Lipids were measured using enzymatic colorimetric methods, while apolipoprotein B was determined using an immunoturbidimetric method. Serum glucose was measured using hexokinase. HbA1c was measured using ion-exchange quantitative high-performance liquid chromatography. CRP was determined with a particle enhanced turbidimetric assay. C-peptide and insulin concentrations were analyzed on frozen samples at the Hormone Laboratory of Oslo University Hospital/Aker, using a non-competitive electro-chemiluminescence immunoassay (ECLIA) (Modular E170 Cobas e601 kit Roche Diagnostics).

In this paper, the surrogate marker of insulin resistance HOMA-IR, based on fasting insulin and glucose measurement was calculated as described above. For confirmation, we performed direct measurements of insulin resistance in a subset of the population using the hyperinsulinemic-euglycemic clamp. Subjects attended the clinic in a fasting state, venous catheters were placed in both arms and insulin (300 mU x ml^{-1}) was infused at a rate of 40

mU x m^{-2} x min^{-1}. Plasma glucose was measured every 5 minutes and kept constant at 5 ±0.5 mmol/l with a variable infusion of glucose 200 mg x ml^{-1}. Total clamp duration was 150 minutes. During the last 30 minutes of the clamp, serum insulin was measured every 10 min and the glucose infusion rate was calculated from the last 30 minutes of stable euglycemia and was expressed in µmol x m^{-2} x min^{-1}. Due to lack of resources, the clamp was only performed in 20 individuals at randomization and at the end of the study.

6 Ethics and approvals

The research was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained by the study physician before any study procedures were performed. The Regional Committee for Medical and Health Research Ethics of Norway evaluated the studies. (Ref.nr. 204-04-03236, from 22.04.2004, Papers I-III). The RCT (Paper IV) was registered at www.clinicaltrials.gov NCT02589756. The study was evaluated by the Ethics committee for region 1 in Norway (Ref.nr. 2015/1930, from 01.10.2015). The upper BMI border was extended to 38kg/m^2 in order to attain adequate recruitment within a reasonable time limit. This change was approved by the Ethics committee with additional (Ref.nr. 2015/1930, from 23.03.2017).

7 Statistical analyses

7.1 Sample size and calculations

Power calculations were not performed for papers I-III given the cross-sectional design and the inclusion of several hundred participants from clinic referrals. Power calculations were performed for the RCT reported in Paper IV. We used the data from Nurses' Health Study, because similar studies to the current one have not been performed previously to help in calculation of sample size (105). Based on published data from this study, we designed the clinical trial to show a difference in POPs between the intervention groups and controls: Median HCB level of about 30 ng/g lipids, a difference of a mean of 36 ng/g lipids between groups with a standard deviation of about 9 ng/g lipids would require 36 participants in each group, with power set at 80% and alpha set at 5% We therefore calculated that we need to include 40 participants in each group to allow for dropouts for a total of 120 participants.

7.2 Statistical analyses

Statistical analyses in all papers were performed using IBM SPSS Statistics for Windows, version 21 (SPSS Inc., Chicago, IL). The significance level was set at $p<0.05$. Continuous data are presented with mean value and standard deviation, mean value with confidence interval or median with 25^{th} and 75^{th} percentile (variables with no normal distribution). In papers I-III, one-way analysis of variance (ANOVA) was performed to analyze difference in 5 groups of metabolic syndrome components. Partial correlations were performed as follows: Paper I: Between PYY3-36 and risk factors; Paper II: Between BMI and waist circumference and metabolic syndrome components and other biomarkers; and Paper III between sums of POPs and BMI and total cholesterol. In paper I we performed independent t-tests to compare components of metabolic syndrome and other risk factors

across groups, as well as linear regression analysis to establish independent relationships between PYY3-36 concentrations and risk factors that showed statistically significant partial correlations. In paper III we also analyzed age-adjusted mean of POPs and calculated the 75th percentile of each organochlorine pesticide and the sum of organochlorine pesticides was calculated separately for each sex. The same procedure was performed for the sum of dioxin-like PCBs, and non-dioxin-like PCBs, and we examined the data for U-shaped associations by examining the mean of each metabolic syndrome component and its markers according to tertiles of each organochlorine pesticide and tertiles of organochlorine pesticide, dioxin-like and non-dioxin-like PCB sums. Bivariate correlations between POPs and age were performed and finally binary regression model was used.

In paper IV we followed the intent-to-treat principle with the last value carried forward for dropouts, with additional complementary analyses of the per protocol population. Independent Student's t-test was performed comparing fatty fish group and nut group with controls. Variables that were not normally distributed were presented as median and 25th and 75th percentile and changes were analyzed using the Mann-Whitney test.

7.3 Missing data

In paper I, we compared the subgroup that lacked PYY values (n=172) with included participants (n=291). Mean BMI was similar in both groups (~46 kg/m^2; SD~5). Mean age in excluded participants was 42 years (SD 13) while in included mean age was 41 years (SD 11); (t=1.3, p=0.3). Of those with missing values, 70% were women compared to 62% in the included group (Chi^2 2.3, p=0.08) and 24% versus 23% were smokers, respectively (Chi^2 0.04, p=0.8).

In paper II, we compared the subgroup that was excluded due to missing values relevant for metabolic syndrome (n=53) with included participants (n=547). Of those that were excluded, 56% were women and 40% were smokers compared to 63% that were women

and 24% smokers in included participants (Chi^2 1.4, p=0.2; Chi^2 7.0, p=0.008, respectively). Mean BMI in those excluded was 43 kg/m^2 (SD 6) and age 42 years (SD 12), compared to included participants with mean BMI 42 kg/m^2 (SD 5), and age 40 years (SD 12) which did not differ (t=1.4, p=0.2; t=1.2, p=0.2, respectively).

Participants without adequate samples for analysis of POPs were excluded from paper III (n=116), 64% of them were women and 22% were smokers, while in included participants (n=431), 63% were women and 25% smokers (Chi^2 = 0.05, p=0.8; Chi^2 =0.5, p=0.5, respectively). Mean BMI was 43 kg/m^2 (SD 6) and mean age 40 years (SD 13) in excluded participants, while mean BMI was 42 kg/m^2 (SD 5) and mean age 40 years (SD 12) in included participants (t =1.6, p=0.1; t=0,4 p=0.7 respectively), showing no substantial differences between included and excluded participants.

Missing blood analyses of specific analytes are shown within Papers I-III, and these numbers were small.

In paper IV, number included of screened population was 175 participants, 31 did not meet the criteria for the study and 13 declined to participate after screening. 131 participants were randomized and 120 completed the study.

8 Results

Paper I

The main finding of this study was that fasting PYY_{3-36} concentrations correlated with systolic blood pressure as the only component of metabolic syndrome after adjustment for age and gender in participants with extreme obesity. In addition to partial correlations, linear regression analysis was performed and showed that PYY_{3-36} concentrations were associated with systolic blood pressure after adjustment for age, gender, and central obesity in the entire sample (Beta 0.21; 95% CI 0.09-0.34) as well as in subjects not taking blood pressure-lowering medication (Beta 0.19; 95% CI 0.04-0.36). These associations were not statistically significant in the small subset of participants (22%) with type 2 diabetes. This might be due to smaller selection of participants. PYY_{3-36} concentrations were not related to other metabolic syndrome components, HOMA index or to inflammatory biomarker or leptin concentrations.

Paper II

The main findings of this study were that degree of obesity, measured by BMI or waist circumference, did not predict metabolic syndrome components, except for systolic and diastolic blood pressure in men, and systolic blood pressure in women with morbid obesity, BMI was not associated with number of metabolic syndrome components. Age and the proportion of men increased with increasing numbers of components of metabolic syndrome, and the level of each component of metabolic syndrome increased with increasing numbers of components. Apolipoprotein B, ferritin, uric acid and alanine aminotransferase (ALT) concentrations worsened with increasing metabolic syndrome components in ANOVA analysis ($p \leq 0.0001$) while BMI and LDL-cholesterol showed no association. BMI correlated inversely with triglycerides ($r=-0.16$, $p=0.03$) and positively with HDL-cholesterol in men ($r=0.16$, $p=0.02$) but not in women. BMI correlated with CRP ($r=0.32$, $p<0.0001$; $r=0.24$,

p<0.0001 in men and women, respectively) and white blood cell count (r=0.24, p=0.001 in men; r=0.15, p=0.008 in women). Truncal fat percentage correlated to CRP (r=0.31, p=0.03; r=0.20, p=0.02 in men and women, respectively). In women, number of metabolic syndrome components was inversely related to truncal and peripheral fat (r=-0.20, p=0.02; r=-0.42, p<0.0001, respectively) as was ALT (r= -0.21, p=0.009; r= -0.38, p<0.0001, respectively) and triglycerides with peripheral fat (r= -0.38, p<0.0001) while HDL cholesterol was positively associated with truncal and peripheral fat (r=0.26; p=0.001). BMI and fat distribution showed expected associations to biomarkers of inflammation, but we observed paradoxical relations between fat indices and metabolic syndrome components.

Paper III

The main findings of the study were that the odds of metabolic syndrome was increased by 2.3 times (OR 2.3 [95% CI 1.3-4.0]) in participants with high circulating concentrations of organochlorine pesticides (HCB, β-HCH, *trans*-Nonachlor, p,p'DDT, p,p'DDE), 2.5 times (OR 2.5 [95% CI 1.3-4.8]) in participants with high circulating concentrations of dioxin-like PCBs and doubled in those with elevated non-dioxin-like PCB concentrations compared to those with lower circulating concentrations (OR 2.0 [95% CI 1.1-3.8]) in logistic regression analysis adjusted for age, gender, BMI, smoking, alcohol consumption and total cholesterol concentration. Age-adjusted concentrations of *trans*-Nonachlor, dioxin-like and non-dioxin-like PCBs increased with number of metabolic syndrome components in both genders (p<0.001), while the organochlorine pesticides HCB, β-HCH and p,p'DDE increased only in women (p<0.008)**.** Organochlorine pesticides were associated with HDL cholesterol and glucose (OR=2.0 [95% CI=1.1-3.4]; 2.4 [95% CI=1.4-4.0], respectively). Dioxin-like PCBs were associated with diastolic blood pressure, glucose and homeostatic model assessment-insulin resistance index (OR=2.0 [95% CI=1.1-3.6], 2.1

[95% CI=1.2-3.6] and 2.1 [95% CI=1.0-4.3], respectively). These results suggest that higher concentrations of POPs increase risk of metabolic syndrome in morbidly obese individuals.

Paper IV

The main finding in paper IV was that there was no change in circulating concentrations of 15 POPs during 6 months of high dietary intakes of fatty fish compared to avoidance of fatty fish in the diet among persons with overweight or obesity and risk of cardiometabolic disorders. Changes in concentrations of all individual and classes of POPs did not differ between the dietary groups ($p>0.05$). Among cardiometabolic risk factors HDL-cholesterol increased in the fatty fish group compared to controls (increase of 0.10 mmol/L, CI [0.05-0.20]; $p=0.005$). Results of the hyperinsulinemic-euglycemic clamp analyses were not shown in the published paper due to lack of space. We observed no changes in glucose infusion rate in participants in the fatty fish and control groups. Glucose infusion rate in fatty fish group showed a change of 0.04 µmol x m^{-2} x min^{-1} (SD 1.1), [4.5 µmol x m^{-2} x min^{-1} (SD 2.3); 4.5 µmol x m^{-2} x min^{-1} (SD 2.3) baseline, end of the study, respectively] and in controls -0.3 µmol x m^{-2} x min^{-1} (SD 2.0), [5.3 µmol x m^{-2} x min^{-1} (SD 2.0) 5.1 µmol x m^{-2} x min^{-1} (SD 2.4) baseline, end of the study, respectively]; $p=0.7$ between groups.

9 Discussion

9.1 Methodological discussion

9.1.1 Participants and selection bias

Papers I-III were based on a previously collected dataset including consecutive patients referred by their general practitioner or by a medical specialist to the Preventive Cardiology Clinic at Oslo University Hospital, Oslo, Norway. In paper I, we only included participants with extreme obesity, with or without comorbidities, as this subgroup has only been studied to a limited degree previously in relation to their cardiometabolic risks. In paper II, participants with morbid obesity were included if they had one or more components of metabolic syndrome (waist circumference was as expected elevated in all) in order to study individuals with metabolic risk. Participants from paper II were included in paper III, if they had banked blood samples that were used in analysis of POPs.

For paper IV we recruited healthy volunteers with BMI between 25 and 38 kg/m^2. Most participants were respondents to newspaper advertisements. A few of the patients were treated previously at the Preventive Cardiology Clinic and gave informed consent to participation in the study.

Selection bias is a distortion of the results based on selection of the study participants leading to conclusions that do not accurately represent the target population to which they are being extended. This usually occurs because of bias in selecting participants for the study (115). Participants in papers I-III were consecutively referred to the clinic and almost all agreed to participate, as describe previously. They were mainly referred from their general practitioner for treatment for overweight, obesity and comorbidities. We suggest that they may be representative for the population in general though there may be a question as to representation of ethnic groups. We estimated that this clinic-based obesity population

included ~ 9-10% non-ethnic Norwegians (based on names). Thus, this group was at least partially represented. However, the proportion of inhabitants of non-ethnic Norwegian descent in Oslo is over 30% and in Viken is over 15%. We did not perform subgroup comparisons of this group due to their limited numbers.

Statistical analysis of the excluded participants from papers I-III showed no substantial differences from included participants regarding main anthropometric characteristics. The main exception is those included in papers II and III where those with missing criteria for metabolic syndrome were more likely to be smokers. This seems to be in line with for example studies on mass-screening programs in Europe and Australia (116, 117). In these studies smokers were more likely to not participate in screenings compared to non-smokers, possible because smokers may underestimate unhealthy health behaviors (118).

In paper IV participants may be defined as more or less healthy overweight or obese people, possibly more interested in research, and many of them with good cultural resources, or "healthy volunteers". Participants were approximately equally divided according to gender and selected regarding the presence of overweight or obesity and at least 1 other cardiometabolic risk factor. The participants probably were representative of the population with cardiometabolic risks given the wide inclusion criteria, though we do not have a population-based comparative group.

9.1.2 Study design

Papers I-III were based on cross-sectional designs. A cross-sectional study is a form of observational study, where factors such as changes in lifestyle or environment or unmeasured confounders cannot be completely assessed. The advantage of cross-sectional studies is that they can be conducted with low expense, and many participants can be recruited. However, cross-sectional studies cannot conclude regarding cause and consequences of risk or disease.

In our studies we were limited to single measurements for all laboratory analyses and physical characteristics including blood pressure, which may be affected by several factors (stress, meals the previous day, physical activity). Thus, the measures are moment specific and may fluctuate over time.

In paper I only participants with extreme obesity were included with no control group, due to lack of a normal weight or less obese population for comparison. In paper II, individuals with BMI ≥ 35 kg/m^{2}were included to better understand the relations between obesity and metabolic syndrome. Again, a weakness was lack of a less overweight population as a comparison.

Paper IV was a randomized clinical trial where participants were allocated at random to receive 1 of 3 dietary interventions and followed prospectively. Paper IV had several strengths, among them the randomization procedure with stratification for gender and BMI resulting in approximately equally distribution of participants according to gender and BMI. Participants had to be willing to be assigned to any of the three dietary groups. Participants were randomized by strata that were produced by an independent statistician. Strata lists were placed in an envelope which was opened by an independent patient secretary that did not otherwise participate in the study. We used 4 different strata defined as follows: women with BMI between 25.0 kg/m^2 and 29.9 kg/m^2, women with BMI between 30.0 kg/m^2 and 38.0 kg/m^2, and men with BMI between 25.0 kg/m^2 and 29.9 kg/m^2 as well as men with BMI between 30.0 kg/m^2 and 38.0 kg/m^2. Strata included randomization number and a group code (F for fatty fish, N for nuts, and C for controls). Compliance was assured at every visit and was controlled in the FFQs. A major limitation of the study is that we could not perform a blind or double-blind RCT, because of the nature of dietary studies. Furthermore, analysis of the data was not done blindly as the doctoral student's responsibility included both following participants and analyzing the data.

9.1.3 Information and confounding biases

Errors can occur in all phases of research and consequently lead to measurements that are different from true values and can lead to false conclusions. These errors are called bias (119). Different types of bias, especially those that are relevant in this thesis include information and confounding biases.

Information bias relates to information given by the participants. In papers I-III information bias can be present in regard to smoking status or other lifestyle factors, because the information was based primarily on self-report. A specific type of information bias, also called recall bias may be defined as systematic errors when participants do not remember previous events accurately, often related to characteristics of the exposure of interest of the respondents (120). Information bias may be present in data on diet or alcohol consumption, as examples. We did not have data on physical activity and dietary data in papers I-III. On the other hand, recall bias regarding diet may be present in paper IV, where participants filled out food frequency questionnaires. This occurred firstly, at randomization, where they were asked to describe their diet 1 year back in detail. And this could have occurred at the end of the study, when describing diet during the study. Also, the complexity of the FFQ may have led to this type of recall bias. Some of the participants did not fill out their questionnaires at the end of the study, which may be defined as non-response bias, also a part of information bias. Lately, it appears that error terms for certain nutrients may be much larger in FFQ data than in food diary data (121).

Confounding bias is defined as ‘’mixing of effects’’ due to an additional, usually not known parameter resulting in a distortion of the true associations (122). In paper I we performed linear regression model and controlled for age, gender and central obesity, as well as use of a blood pressure lowering medication. In paper II we controlled for age, gender and

smoking status. In paper III we first calculated age-adjusted means of POPs concentrations. In multiple logistic regressions we controlled for age, gender, BMI, smoking status, alcohol consumption and cholesterol concentrations. However, cross-sectional studies will generally retain confounding bias despite control for known confounders.

In paper IV we used a randomized controlled design to avoid bias and a randomization procedure with stratification for gender and BMI. The study was conducted prospectively, with changes in risk factors and POPs in 2 intervention groups compared to a control group that avoided fatty fish or nuts consumed in the intervention groups.

9.1.4 Internal and external validity

Internal validity refers to the utility of the results valid for the studied population (123). Internal validity is how well a study is done, particularly whether it avoids confounding, with other words, internal validity is higher when there is less confounding (124). Internal validity in paper IV is strong, because participants were encouraged to maintain a stable lifestyle and did not fluctuate in body weight. However, we did not collect data on physical activity.

External validity refers to the utility of the results end implementation in populations outside the study (123). The question is whether the results may be valid for other overweight and obese individuals or persons with the same characteristics as in our study. Participants from papers I-III are probably representative of the treatment-seeking part of the obese population living within reasonable distance from the clinic. Some of the referred participants may have not been interested in treatment but were referred to the hospital clinic for evaluation and to increase their motivation (personal observations). This type of recruitment may also be a disadvantage, because it may affect patients' motivation positively, and may have implications on some results. Population from paper IV represent obese people with

possibly higher cardiovascular risk due to presence of at least 2 parameters of metabolic syndrome (waist circumference and 1 more) than the general obese population, and the results may be valid in obese people with similar cardiometabolic risk.

9.2 Surrogate markers of cardiometabolic risk

Surrogate markers can be defined as ‘’a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the therapy”, and these biomarkers must be separated from measurements that reflect the activity of a disease (125). Such markers may be causal of disease, but are often mediators, regulators or reporters of disease (quoted from a webcam with Professor Chris Packard). Anthropometric measurements, including waist circumference and BMI have been traditionally used in defining cardiometabolic risk and are highly correlated. Laboratory analyses such as lipids and lipoproteins (triglycerides, LDL cholesterol, apolipoprotein B), glucose, inflammatory markers (ferritin, CRP) are in wide practical use. Some are causal (LDL cholesterol) while others are mediators (CRP). For instance, a study based on mendelian randomization showed that CRP is not a causal factor of CHD (126). Some surrogate markers may have limitations due to fluctuations and many parameters influencing it. An example would be insulin levels. Potential good surrogate endpoints in studies should assure information of causality, and result in endpoints that provide better live quality, longevity, and are at reasonable cost (127). Not all surrogates have this capacity.

9.2.1 Markers of metabolic syndrome and insulin resistance

Metabolic syndrome is tightly associated with insulin resistance. Some markers may indicate metabolic syndrome better than others. For instance, C-peptide is suggested as a better surrogate marker of CVD in diabetes patients than insulin, due to less measurement

variability and given its effect on smooth muscle cell proliferation and inducing atherosclerosis (128). C-peptide is produced in equimolar amounts to insulin and has traditionally been used as β-cell functional marker, because of its stable concentration in circulation and insignificant hepatic clearance, while about ½ of secreted insulin is metabolized by first-pass through the liver (129). C-peptide measurements are cheap, facilitating measurements in routine clinical practice, and non-invasive (130). C-peptide is a good parameter of insulin secretion, but not its function (113).

Taken from the broader perspective, there are several other indices of metabolic syndrome than those that are not part of the strict definition, such as CRP levels, other markers of subclinical inflammation, fatty liver, polycystic ovary syndrome and others. These markers tend to be correlated with each other but may represent different aspects of the condition. Elevated CRP is prevalent even among individuals with metabolically healthy obesity, and may contribute to identifying the risk of adverse outcomes in this group (131). White blood cell counts have been also been connected to metabolic syndrome (132). We found that the white blood cell count correlated with both BMI and waist circumference in both men and women (57). Fatty liver may be indicated by elevated transaminases, which are correlated with ferritin and uric acid concentrations. These are all associated with severity of metabolic syndrome (133, 134). Apolipoprotein B correlates with metabolic syndrome, independently of LDL-cholesterol levels (135), which is in line with our results.

9.2.2 The Homeostatic Model Assessment for Insulin Resistance

Another indicator of metabolic syndrome is the Homeostatic Model Assessment for Insulin Resistance index (HOMA-IR), which increases significantly with increasing number of metabolic syndrome components and it is still an easily calculated parameter of insulin resistance and metabolic syndrome (136), and it is a widely used epidemiological and clinical

method for assessing insulin resistance (IR) from fasting glucose and insulin or C-peptide concentrations (113). The notion of defining HOMA-IR index is based on the inappropriate β-cell response to raising plasma glucose concentrations, in individuals with insulin resistance, or decreasing the effects of plasma insulin concentrations at both the liver and the peripheral tissues (137). The originally suggested HOMA-IR index was calculated and simplified (113), which is the calculation used in the current studies. HOMA-IR index needs to be interpreted carefully, as it is a surrogate marker of insulin resistance. Furthermore, it rarely shows a normal distribution, which requires logarithmic transformation (113). HOMA-IR index is based on fasting insulin levels and glucose, and thus does not describe post-prandial effect of insulin, and furthermore does not differ between hepatic and peripheral insulin resistance (138).

9.2.3 Hyperinsulinemic-euglycemic clamp

The hyperinsulinemic-euglycemic clamp described in 1979 by DeFronzo and colleagues (139), has become the gold-standard method to assess insulin sensitivity today, and is widely used in clinics and laboratories to measure insulin action on glucose utilization in humans for clinical research (140). However, the method is expensive and time-consuming and requires high human resources as well. Our data on clamp are modest, and we did not see any change in glucose infusion rate in the 2 groups. The sample size is the main limitation for this clamp analysis. The clamp procedure was performed in an animal model and showed that exposure to POPs from the diet leads to insulin resistance (141). In a human model, individuals that were examined with this method in a cross-sectional study showed that prediabetic and diabetic patients had higher levels of POPs in comparison of normoglycemic individuals (142). Thus, this method is relevant to study of POPs. However, we cannot draw any material conclusions from our clamp analyses due to the limitations mentioned above. A sample size calculation based on a glucose infusion rate of 6 (SD 2) μmol x m^{-2} x min^{-1} in the

group consuming fish versus 20% less in the control group would require that the procedure be completed in all the participants (ClinCalc.com).

9.3 Categorization of POPs

As very many different types of POPs are found and have been studied, we grouped them according to their chemical structure or origin and use as follows.

Organochlorine pesticides are chlorinated hydrocarbons, which were in use in agriculture and mosquito control, especially during 1940-1960, but are still present in soil today. One of the most known pesticides is p,p'DDT or commonly known as DDT. PCBs were used in electrical equipment or hydraulic fluids, heat transfer fluids (coolants), and could be released into the environment through, leaks from electrical equipment. As the other organic pollutants, they can be transferred long distances in the environment. They can? be found in air, water, soil, and sediments throughout the world. In paper III, we analyzed POPs, mainly organochlorine pesticides and dioxin-like PCBs and non-dioxin-like PCBs. All organochlorine pesticides with the exception of p,p'DDT, increase with number of metabolic syndrome components, and in men, dioxin-like and non-dioxin-like PCB concentrations increased according to number of metabolic syndrome components. High concentrations of dioxin-like PCBs and non-dioxin-like PCBs are associated with metabolic syndrome and some of its components in morbidly obese people, and this might be a first study that explores these relations. Both organochlorine pesticides and PCBs were related to glucose metabolism and type 2 diabetes in previous studies (143, 144). Previous research suggests that persistent PCBs with ≥ 7 chlorines may be more predictive than other PCBs (104).

9.4 Discussion of results and limitations

9.4.1 Adipokines and gastrointestinal hormones in patients with extreme obesity

Our main findings from paper I were that fasting concentrations of the PYY_{3-36} did not correlate with the number of metabolic syndrome components. The sole exception was the correlation between PYY_{3-36} and systolic blood pressure in the entire sample and in participants not taking blood pressure lowering medication. The other markers of metabolic disturbances or inflammatory biomarkers or lipids (LDL cholesterol, apolipoprotein B), leptin concentrations as well as HOMA-IR index were not related to this gastrointestinal hormone in extremely obese subjects. Our main findings of no relation between PYY_{3-36} concentrations and metabolic syndrome components, insulin resistance or presence of type 2 diabetes differ from some published studies. A study published in 2015 suggested that in patients with coronary artery disease, not selected for obesity, high concentrations of PYY_{3-36} were associated with type 2 diabetes and insulin resistance, as well as with obesity (145). The same study found associations between high concentrations of PYY_{3-36} and high fasting glucose, but the same association was not found when BMI was removed as confounder (145). Our findings were like other studies that studied extremely obese individuals (146). Some authors speculated about possible connections between insulin, leptin and PYY resistance (shown by elevated levels). Fasting PYY_{3-36} concentrations were not related to other inflammatory markers (CRP, ferritin, and white cell count) in our study. Our main findings may be consistent with an animal study that showed vasoconstrictive effect of the hormone, and its presence in the atherosclerotic plaque in the same vessels (147). Another study from 1986 showed that intravenous infusion of PYY caused significant vasoconstriction (148).

PYY_{3-36} is a hormone of clinical relevance. Weight loss after Roux-en-Y gastric bypass showed that concentrations of PYY_{3-36} hormone did not differ between participants with and without weight reduction after bariatric surgery (149), but some authors have suggested the opposite, that following gastric bypass surgery anorectic gut hormones, such as PYY and GLP-1, are elevated and play crucial role in postprandial satiety in those patients.

PYY has been considered to have the potential of playing a role as a pharmaceutical treatment of obesity, but may be most effective if administered in combination with other substances (32).

The most important limitation of paper I is that we only had a single pre-prandial PYY_{3-36} measurement. A study showed that postprandial PYY concentrations are positively associated with gray matter volume bilaterally in the caudate nuclei, and may modulate eating behavior via striatal networks (150), unfortunately we did not have these measurements. Further, we did not compare our results with those in individuals with lower levels of obesity, or lean individuals. Blood pressure was measured at clinical visit only as a single occasion, and it would be useful to compare these results to results that could be seen with an ambulatory 24-hour blood pressure measurement.

9.4.2 Metabolic syndrome in patients with morbid obesity

The main finding of paper II was that the degree of obesity, either measured by waist circumference or BMI was not a predictive factor of metabolic syndrome components in women with morbid obesity, except for systolic blood pressure. On the other hand, in men within same obesity degree, both systolic and diastolic blood pressures were positively correlated with these 2 markers of obesity, but we also observed reciprocal relation of BMI and lipids. Further, our findings showed decreasing amount of total and truncal fat and at the same time increasing number of metabolic syndrome components in both genders, while the same effect was observed for peripheral fat only in women. In this study 1/3 of population were men, and all results were controlled for age and smoking. The fact that BMI or waist circumference did not correlate with the number of metabolic syndrome components may suggest that other factors determine metabolic syndrome in morbid obese individuals, such as hepatic fatty infiltration as described in other studies (133) or heredity or other unmeasured

factors. Previous studies included limited samples of severely obese individuals (151-153). Further, waist circumference was weakly associated with number of metabolic syndrome components in our study, and only in women. These findings may suggest that waist is more useful in determining of metabolic syndrome in less obese or non-morbidly obese individuals, than those who are morbidly obese. CT scan visualization would be of higher interest than the dual-energy x-ray absorptiometry (DXA) method we used.

More than 2/3 of our sample met the metabolic syndrome criteria (41). The rest of the sample were ‘’metabolically healthy’’ but obese individuals (34), and most of them were women of younger age. The latter observation indicates that they may not be permanently protected from metabolic disturbances (154).

As expected, CRP was correlated with BMI and waist circumference, both in men and women. The white blood cell counts correlated with the same markers but were not elevated in the same grade as highly sensitive-CRP. Even though these inflammatory biomarkers did not correlate with severity of metabolic syndrome, CRP showed associations with total, truncal and peripheral fat depots. These findings are consistent with studies that showed that subclinical inflammation is important indicator of metabolic risks of obesity (134, 155).

We studied liver dysfunction and oxidative stress markers (ferritin, uric acid, ALT). Ferritin and uric acid concentrations were associated with severity of metabolic syndrome, which suggest that they can be useful markers of metabolic disturbances, as shown in other studies (134). ALT concentration is the indicating factor of liver dysfunction and is traditionally associated with severity of metabolic syndrome and waist circumference in women. Our reciprocal associations between ALT and fat depots in morbidly obese women may suggest protective role of fat depots, but this is not possible to conclude in a cross-sectional study.

Dyslipidemia characterized by high triglycerides, low HDL cholesterol and small dense LDL particles in overweight or obese individuals is well documented (156). Lipolysis of triglyceride-rich lipoproteins is reduced, accelerating the development of small dense LDL particles, low HDL cholesterol levels and small dense HDL particles and relatively high apolipoprotein B levels. However, we found no relation between BMI or waist circumference and lipids in women, while in men; relationships were paradoxical, with positive relations between BMI and HDL-cholesterol and an inverse relationship between BMI and triglycerides. There is a possibility of sequestering of fat in adipose tissue in morbid obesity which gives some explanation of our paradoxical findings (157). Overall, we may speculate that extremely obese individuals may not exhibit the same dysregulation of lipids as individuals with less obesity.

A limitation of this study is selection of the participants which was solely a morbid obese population, where all participants had BMI $\geq$ 35 kg/m^2. We did not have a lean or less overweight population for comparison. Further we lacked dietary, alcohol consumption and physical activity data of the participants. Less than 1/2 (38%) of participants completed DXA measurements, due to limitations of DXA machines in measuring very heavy individuals weighing above 120 kg.

9.4.3 POPs in patients with morbid obesity

Paper III was a cross-sectional study conducted in morbidly obese men and women, based on the same population as Paper II, but including only those participants that had available blood samples in the biobank for the analysis of POPs. To our knowledge, this is the first study to explore the relation between POPs concentrations and metabolic risks in the morbidly obese individuals. The main findings were that odds of metabolic syndrome were increased by 2.3 times in participants with high circulating concentrations of organochlorine

pesticides, 2.5 times in participants with high circulating concentrations of dioxin-like PCBs and doubled in those with elevated non-dioxin-like PCB concentrations compared to those with lower circulating concentrations.

In participants with elevated organochlorine compounds, the concentrations of HDL-cholesterol was decreased, which was also observed in a prospective study of 10 top ranked POPs, including hexachlorobenzene (158), while fasting glucose concentrations were elevated which is in line with the definition of metabolic syndrome (41). In participants with elevated dioxin-like PCBs, diastolic blood pressure and fasting glucose concentrations were elevated, as was HOMA-IR, indicating insulin resistance. This seems to be in line with other studies, that organochlorine pesticides have been shown to increase risk of hypertension in overweight Spanish subjects over a 10-year follow-up period (159). The highest concentrations of POPs were also associated with systolic and diastolic blood pressure in linear regression tests in persons with hypertension, when adjusted for covariates, while in normotensive subjects blood pressure was related to the top tertile POPs concentration (160).

The population studied may represent a higher cardiovascular risk population than the general obese population, given their referral for treatment. Despite likely selection bias, over 25% of men and 40% of women did not meet the definition of metabolic syndrome, as they demonstrated only 1-2 components, including waist circumference, in all cases, and none or only 1 more metabolic syndrome component.

Age-adjusted concentrations of POPs increased with number of metabolic syndrome components, and they were strongly age-related. Lipophilic compounds accumulate in fat deposits with time and older patients were exposed to high exposures at a time when environmental levels were higher than today.

Studies in non-diabetic subjects have found associations between POPs and dyslipidemia and metabolic syndrome or high fasting glucose concentrations (161). As mentioned before, POPs were grouped into three categories, according to their chemical structure and origin or use (organochlorine pesticides, dioxin-like PCBs and non-dioxin-like PCBs). In addition both of these were shown to have an effect on metabolic syndrome as well, but synergistic effects were not found (158). They were also connected to the other metabolic disturbances (104), such as fasting glucose. We did not find a significant association between waist circumference and organochlorine pesticides in morbidly obese individuals, but some studies have such findings with organochlorine pesticides (161, 162).

We found that dioxin-like and non-dioxin-like PCBs were weakly, and not significantly inversely associated with BMI. A dilution effect of very high BMIs on POPs has been described. When absorption of POPs exceeds elimination rate and this might be the case (163).

We found an inverse relation between concentrations of non-dioxin-like PCBs and CRP concentrations in men. Similar results were observed in a cross-sectional study of non-diabetic participants in the United States (164). In our study only organochlorine pesticides were positively associated with CRP concentrations. CRP levels were associated with HOMA-IR only in subjects with high POPs, possibly indicating effect modification by POPs.

The main limitation of the study is that we did not have data regarding dietary habits or physical activity. Further, we did not normalize POPs concentrations for lipid values, though we controlled for cholesterol concentrations in regression analyses. Normalization for lipid concentrations is controversial (165). We did control analyses with controlling for lipids, and this did not change our results substantially. Levels of POPs that we used are circulating POPs levels, which possibly may differ from levels in adipose tissue and liver.

It is possible that subcutaneous adipose tissue has a lesser storage capacity than visceral adipose tissue which may result in increased lipolysis and release of free fatty acids (166, 167).

9.4.4 Effect of diet on POPs in patients with overweight and obesity

Paper IV was a randomized controlled clinical trial. The main findings were that circulating concentrations of 15 POPs during 6 months of high dietary intakes of fatty fish did not increase compared to the group that avoided fatty fish among persons at higher risk of cardiometabolic disorders. We also included a group consuming nuts and avoiding fatty fish, but we did not find any differences between these groups either. Only HDL-cholesterol increased in the group consuming fatty fish. More frequent ingestion of fatty fish showed lower levels of non-HDL cholesterols in a Japanese cross-sectional study from 2020 (168)

The strength of the study is that we used a randomized controlled design to avoid bias and a randomization procedure with stratification for gender and BMI. That means also that we had approximately equally distribution of participants according to gender and selected regarding the level of overweight or obesity in each group. We had a low level of dropouts overall (~8%). Further, we are unaware of any published similar randomized interventional studies that explored POPs concentrations in overweight and obese individuals regarding fatty fish intakes.

We used the same method of classifying POPs as in previous study (organochlorine pesticides and dioxin-like and non-dioxin-like PCBs), based on associations between these groups and cardiometabolic disturbances both in cross-sectional and prospective studies (169, 170). POPs are associated with several cardiometabolic risk factors, and conditions related to overweight and obesity including insulin resistance and related disorders (171). A study that examined Inuits living in Greenland that consume large amounts of fish found that POPs may

adversely affect insulin secretion (172). Elevated p,p'DDE concentrations have been demonstrated in patients with diabetes (173). We did not find changes in HOMA-IR index or in the glucose infusion rate (clamp) between participants in the fatty fish group versus controls (as mentioned previously, only 10 individuals from each group underwent the clamp procedure).

Our results regarding fatty fish consumption and increase in HDL-cholesterol levels supports a study conducted in volunteers that found that fatty fish increased HDL particle diameter and concentrations of lipid components in HDL-cholesterol (174). This finding may indicate that the increase in HDL-cholesterol in the fatty fish group compared to the group consuming nuts reflected the lack of fatty fish in the diet of the group consuming nuts. We did not find a reduction in triglyceride concentrations in the fatty fish group, perhaps because participants' baseline levels were only mildly elevated. Our results support the notion that the overall health benefits of high consumption of fish may outweigh the adverse effect of contaminants (175).

We found no increase in POPs in the nuts group compared to controls in the current study. Nut consumption may lower all-cause mortality and CVD (88). Cohort and interventional trials indicate that some nutrients that are richly found in nuts could be linked to beneficial health effects of nuts (176), for example lower LDL-cholesterol (174) but this was not the case in our randomized trial. A randomized crossover trial found that both fatty fish and walnuts lowered cholesterol and triglyceride concentrations in individuals with normal or mild hyperlipidemia (177). A hypocaloric vegetarian diet did not find any reduction in concentrations of POPs compared to conventional diet, possibly because of mobilization of fat stores in response to a decreased calorie intake (178).

The weakness of the study was the short study period of 6 months, but a longer study duration of 1 year or more that would be more optimal to show changes in POPs concentrations, may raise concern about compliance and lack of motivation in participants.

We are aware of possible recall bias while filling out the FFQ, as the FFQ covered a year at inclusion and the past 6 months at the end of the study. Composition of salmon has been changed due to feed changes from marine-based diet in the early 1990s to a 70% plant based diet at present and has resulted in a lower n-3 fatty acid content of the fish (179). We did not analyze polycyclic aromatic hydrocarbons (PAHs), which are present in vegetable oils that are in use today (180). We did not analyze the content of POPs in the nuts used.

We did not analyze adipose tissue samples, but another study indicated that a consumption of 380 g of farmed salmon weekly did not increase concentrations of some POPs neither in plasma or in adipose tissue, so that serum levels of POPs may be representative for the levels in adipose tissue (181) .

Yu-Mi Lee and Duk-Hee Lee raised some critical aspects to paper IV (182). They argued that serum concentrations of POPs are mainly determined through the release of POPs from adipose tissue into the circulation, especially in obese individuals due to abnormal adipocytes and lipolysis, and not as the absolute amount of POPs from external sources. Further, in their opinion, the RCT may be a disadvantage, independently of study duration. Cross-sectional studies may be a better model, as they capture long-term fatty fish intake. They suggest that lowering risks due to POPs requires better control of toxicokinetics, that could be influenced by lifestyle, rather than avoidance of POPs from external sources. For example serum concentrations of POPs among physically active subjects were significantly lower than in physically inactive, and moderate fat diet with low carbohydrate ingestion, may result in lower POPs concentrations (182).

However, we do not believe that the RCT model is flawed in answering the question about diet and POPs; furthermore, cross-sectional studies based on food questionnaires covering a long period of time, may be fraught by uncertainties and bias (121). A study that authors cited shows that diet affects POPs concentration. They refer also to animal studies, which differ substantially from human clinical trials. Current dietary recommendations suggest that pregnant women should avoid fatty fish, due to the effect of diet on POPs concentrations for the pregnancy period of 9 months indicating that even short term intakes may influence risk Our findings cannot be extrapolated to how consumption of other fish sources with longer exposure may affect POPs exposure in humans, and potential health consequences (183).

10 Conclusions

Paper I

In extremely obese patients, fasting PYY_{3-36} concentrations were linked to systolic blood pressure, but not to other components of metabolic syndrome, suggesting divergence between pathways of blood pressure and glucose/body weight regulation.

Paper II

BMI and fat distribution showed expected associations to inflammation biomarkers, but paradoxical relations between fat indices and metabolic syndrome components and biomarkers were seen. This suggests a need for better markers of CVD risk in morbid obesity.

Paper III

In subjects with morbid obesity, metabolic syndrome was related to circulating levels of organochlorine pesticides and PCBs suggesting that these compounds aggravate clinically relevant complications of obesity.

Paper IV

Fatty fish consumption for 6 months did not increase the serum concentrations of POPs in individuals with overweight or obesity and metabolic risk. While this finding appears reassuring regarding farmed salmon longer periods of high intakes require further study.

11 Future research

The field of metabolic syndrome is currently a subject of intensive and long-term study. Since the definition of metabolic syndrome was promoted in the late nineties, many studies were conducted to analyze and describe the mechanisms and hazardous effects of metabolic syndrome on especially CVD and CHD.

On the other hand, it is still unknown why some individuals remain metabolically healthy despite to overweight or obesity. Genetics may be important factor. Further, duration of overweight and obesity affect whether a person develops metabolic disorders. We still search for adequate treatment for obesity, some gastrointestinal hormones have shown good results (GLP-1), but PYY_{3-36} may have side effects such as hypertension. We need larger and longer RCTs to examine these effects in a long term. Metabolic syndrome does not need to correlate with BMI, but further analyses including also lean population need to be done.

The effect of our environment on what we eat has attracted increasing attention in more recent years. POPs appear to play an important role in the development of metabolic disturbances. They may be potential disruptors and triggers of metabolic alteration. The standard recommendations for diet and physical activity have not shown great success rates in reducing incidence of obesity and metabolic disturbances. One of the aspects requiring more attention may be the effect of pollutants in the food supply.

Knowledge about relations between diet, metabolic disturbances, pollution and satiety hormones is still poor, and we need more clinical trials to understand them better. These trials may be difficult to conduct, to recruit participants, and find data sources.

We need studies in various patient groups, longer studies and better understanding of diets implications of POPs on human health in order to answer questions like, what is the primary cause of POPs accumulation in body and what is the time perspective for hazardous

effects of POPs? These questions may first require more animal studies before attempting more human studies. In addition to studying the effects of diet, we need a better understanding of physical activity and its effect on POPs accumulation, alone or combined with diet recommendations.

Production of Norwegian salmon has changed from using animal foods and oils to vegetable foods, but this may increase levels of other pollutants in the fish. We still do not have enough data about newer pollutants' possible implications on human health.

How to provide safe foods for our populations? Climate changes and pollution are increasing phenomenon, even though most POPs are banned or restricted, they are still present in the nature, and new types are produced.

12. References:

1. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. https://wwwwhoint/news-room/fact-sheets/detail/obesity-and-overweight. 2018.
2. https://www.who.int/dietphysicalactivity/childhood/en/.
3. Ginsberg HN, MacCallum PR. The obesity, metabolic syndrome, and type 2 diabetes mellitus pandemic: Part I. Increased cardiovascular disease risk and the importance of atherogenic dyslipidemia in persons with the metabolic syndrome and type 2 diabetes mellitus. J Cardiometab Syndr. 2009;4(2):113-9.
4. https://www.fhi.no/en/op/hin/lifestyle/overweight-and-obesity-in-norway---/.
5. Midthjell K, Lee CM, Langhammer A, Krokstad S, Holmen TL, Hveem K, et al. Trends in overweight and obesity over 22 years in a large adult population: the HUNT Study, Norway. Clin Obes. 2013;3(1-2):12-20.
6. Kolsgaard ML, Andersen LF, Tonstad S, Brunborg C, Wangensteen T, Joner G. Ethnic differences in metabolic syndrome among overweight and obese children and adolescents: the Oslo Adiposity Intervention Study. Acta Paediatr. 2008;97(11):1557-63.
7. Schwartz MW, Seeley RJ, Zeltser LM, Drewnowski A, Ravussin E, Redman LM, et al. Obesity Pathogenesis: An Endocrine Society Scientific Statement. Endocr Rev. 2017;38(4):267-96.
8. Kennedy GC. The role of depot fat in the hypothalamic control of food intake in the rat. Proc R Soc Lond B Biol Sci. 1953;140(901):578-96.
9. Speakman JR, Levitsky DA, Allison DB, Bray MS, de Castro JM, Clegg DJ, et al. Set points, settling points and some alternative models: theoretical options to understand how genes and environments combine to regulate body adiposity. Dis Model Mech. 2011;4(6):733-45.
10. Scannell Bryan M, Argos M, Pierce B, Tong L, Rakibuz-Zaman M, Ahmed A, et al. Genome-wide association studies and heritability estimates of body mass index related phenotypes in Bangladeshi adults. PLoS One. 2014;9(8):e105062.
11. Thaker VV. Genetic and Epigenetic Causes of Obesity. Adolesc Med State Art Rev. 2017;28(2):379-405.
12. Bouret S, Levin BE, Ozanne SE. Gene-environment interactions controlling energy and glucose homeostasis and the developmental origins of obesity. Physiol Rev. 2015;95(1):47-82.
13. Gardner R, Feely A, Layte R, Williams J, McGavock J. Adverse childhood experiences are associated with an increased risk of obesity in early adolescence: a population-based prospective cohort study. Pediatr Res. 2019;86(4):522-8.
14. Al-Hazzaa HM, Abahussain NA, Al-Sobayel HI, Qahwaji DM, Musaiger AO. Lifestyle factors associated with overweight and obesity among Saudi adolescents. BMC Public Health. 2012;12:354.
15. Rajan TM, Menon V. Psychiatric disorders and obesity: A review of association studies. J Postgrad Med. 2017;63(3):182-90.
16. Nihalani N, Schwartz TL, Siddiqui UA, Megna JL. Obesity and psychotropics. CNS Neurosci Ther. 2012;18(1):57-63.
17. Niccolai E, Boem F, Russo E, Amedei A. The Gut(-)Brain Axis in the Neuropsychological Disease Model of Obesity: A Classical Movie Revised by the Emerging Director "Microbiome". Nutrients. 2019;11(1).
18. Nicolaidis S. Environment and obesity. Metabolism. 2019;100S:153942.
19. Schecter A, Colacino J, Haffner D, Patel K, Opel M, Papke O, et al. Perfluorinated compounds, polychlorinated biphenyls, and organochlorine pesticide contamination in composite food samples from Dallas, Texas, USA. Environ Health Perspect. 2010;118(6):796-802.
20. https://www.who.int/topics/obesity/en/.
21. Kitahara CM, Flint AJ, Berrington de Gonzalez A, Bernstein L, Brotzman M, MacInnis RJ, et al. Association between class III obesity (BMI of 40-59 kg/m2) and mortality: a pooled analysis of 20 prospective studies. PLoS Med. 2014;11(7):e1001673.
22. Sturm R, Hattori A. Morbid obesity rates continue to rise rapidly in the United States. Int J Obes (Lond). 2013;37(6):889-91.

23. Dutheil F, Gordon BA, Naughton G, Crendal E, Courteix D, Chaplais E, et al. Cardiovascular risk of adipokines: a review. J Int Med Res. 2018;46(6):2082-95.
24. Kumari R, Kumar S, Kant R. An update on metabolic syndrome: Metabolic risk markers and adipokines in the development of metabolic syndrome. Diabetes Metab Syndr. 2019;13(4):2409-17.
25. D'Souza A M, Neumann UH, Glavas MM, Kieffer TJ. The glucoregulatory actions of leptin. Mol Metab. 2017;6(9):1052-65.
26. Park HK, Ahima RS. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. Metabolism. 2015;64(1):24-34.
27. Jazet IM, Pijl H, Meinders AE. Adipose tissue as an endocrine organ: impact on insulin resistance. Neth J Med. 2003;61(6):194-212.
28. Hussain SS, Bloom SR. The regulation of food intake by the gut-brain axis: implications for obesity. Int J Obes (Lond). 2013;37(5):625-33.
29. Manning S, Batterham RL. The role of gut hormone peptide YY in energy and glucose homeostasis: twelve years on. Annu Rev Physiol. 2014;76:585-608.
30. Price SL, Bloom SR. Protein PYY and its role in metabolism. Front Horm Res. 2014;42:147-54.
31. Cahill F, Ji Y, Wadden D, Amini P, Randell E, Vasdev S, et al. The Association of Serum Total Peptide YY (PYY) with Obesity and Body Fat Measures in the CODING Study. PLoS One. 2014;9(4):e95235.
32. De Silva A, Bloom SR. Gut Hormones and Appetite Control: A Focus on PYY and GLP-1 as Therapeutic Targets in Obesity. Gut Liver. 2012;6(1):10-20.
33. Dusanov S, Brandal K, Heggen E, Tonstad S. Associations of Circulating PYY3-36 Concentrations with Metabolic Syndrome in Extremely Obese Subjects. Metab Syndr Relat Disord. 2016;14(8):410-5.
34. Jung CH, Lee WJ, Song KH. Metabolically healthy obesity: a friend or foe? Korean J Intern Med. 2017;32(4):611-21.
35. Stefan N, Haring HU, Hu FB, Schulze MB. Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications. Lancet Diabetes Endocrinol. 2013;1(2):152-62.
36. Bluher S, Schwarz P. Metabolically healthy obesity from childhood to adulthood - Does weight status alone matter? Metabolism. 2014;63(9):1084-92.
37. Iacobini C, Pugliese G, Blasetti Fantauzzi C, Federici M, Menini S. Metabolically healthy versus metabolically unhealthy obesity. Metabolism. 2019;92:51-60.
38. Gepstein V, Weiss R. Obesity as the Main Risk Factor for Metabolic Syndrome in Children. Front Endocrinol (Lausanne). 2019;10:568.
39. Zheng R, Zhou D, Zhu Y. The long-term prognosis of cardiovascular disease and all-cause mortality for metabolically healthy obesity: a systematic review and meta-analysis. J Epidemiol Community Health. 2016;70(10):1024-31.
40. Renninger M, Lochen ML, Ekelund U, Hopstock LA, Jorgensen L, Mathiesen EB, et al. The independent and joint associations of physical activity and body mass index with myocardial infarction: The Tromso Study. Prev Med. 2018;116:94-8.
41. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.
42. Services USDoHH. https://www.nhlbi.nih.gov/health-topics/metabolic-syndrome. 2019.
43. WHO. https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/. 2000.
44. Khosravi A, Sadeghi M, Barghikar M. Which Components of Metabolic Syndrome have a Greater Effect on Mortality, CVA and Myocardial Infarction, Hyperglycemia, High Blood Pressure or Both? Adv Biomed Res. 2017;6:121.
45. Saklayen MG. The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep. 2018;20(2):12.
46. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol. 2010;56(14):1113-32.

47. Galassi A, Reynolds K, He J. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. Am J Med. 2006;119(10):812-9.
48. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care. 2001;24(4):683-9.
49. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA. 2002;288(21):2709-16.
50. Nilsson PM, Engstrom G, Hedblad B. The metabolic syndrome and incidence of cardiovascular disease in non-diabetic subjects--a population-based study comparing three different definitions. Diabet Med. 2007;24(5):464-72.
51. Li W, Ma D, Liu M, Liu H, Feng S, Hao Z, et al. Association between metabolic syndrome and risk of stroke: a meta-analysis of cohort studies. Cerebrovasc Dis. 2008;25(6):539-47.
52. Sarrafzadegan N, Gharipour M, Sadeghi M, Nezafati P, Talaie M, Oveisgharan S, et al. Metabolic Syndrome and the Risk of Ischemic Stroke. J Stroke Cerebrovasc Dis. 2017;26(2):286-94.
53. Li X, Li X, Lin H, Fu X, Lin W, Li M, et al. Metabolic syndrome and stroke: A meta-analysis of prospective cohort studies. J Clin Neurosci. 2017;40:34-8.
54. Shin JA, Lee JH, Lim SY, Ha HS, Kwon HS, Park YM, et al. Metabolic syndrome as a predictor of type 2 diabetes, and its clinical interpretations and usefulness. J Diabetes Investig. 2013;4(4):334-43.
55. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation. 2005;112(20):3066-72.
56. Ford ES, Li C, Sattar N. Metabolic syndrome and incident diabetes: current state of the evidence. Diabetes Care. 2008;31(9):1898-904.
57. Dusanov S, Heggen E, Tonstad S. Characteristics of Metabolic Syndrome in Morbidly Obese Subjects. Metab Syndr Relat Disord. 2016;14(10):500-6.
58. Han TS, Lean ME. A clinical perspective of obesity, metabolic syndrome and cardiovascular disease. JRSM Cardiovasc Dis. 2016;5:2048004016633371.
59. Sami W, Ansari T, Butt NS, Hamid MRA. Effect of diet on type 2 diabetes mellitus: A review. Int J Health Sci (Qassim). 2017;11(2):65-71.
60. Makris A, Foster GD. Dietary approaches to the treatment of obesity. Psychiatr Clin North Am. 2011;34(4):813-27.
61. Jakicic JM, Davis KK. Obesity and physical activity. Psychiatr Clin North Am. 2011;34(4):829-40.
62. Swift DL, Johannsen NM, Lavie CJ, Earnest CP, Church TS. The role of exercise and physical activity in weight loss and maintenance. Prog Cardiovasc Dis. 2014;56(4):441-7.
63. Carnethon MR. Physical Activity and Cardiovascular Disease: How Much is Enough? Am J Lifestyle Med. 2009;3(1 Suppl):44S-9S.
64. Zhang S, Zhuang X, Lin X, Zhong X, Zhou H, Sun X, et al. Low-Carbohydrate Diets and Risk of Incident Atrial Fibrillation: A Prospective Cohort Study. J Am Heart Assoc. 2019;8(9):e011955.
65. Gepner Y, Shelef I, Schwarzfuchs D, Zelicha H, Tene L, Yaskolka Meir A, et al. Effect of Distinct Lifestyle Interventions on Mobilization of Fat Storage Pools: CENTRAL Magnetic Resonance Imaging Randomized Controlled Trial. Circulation. 2018;137(11):1143-57.
66. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. N Engl J Med. 2018;378(25):e34.
67. Eilat-Adar S, Sinai T, Yosefy C, Henkin Y. Nutritional recommendations for cardiovascular disease prevention. Nutrients. 2013;5(9):3646-83.
68. Esposito K, Kastorini CM, Panagiotakos DB, Giugliano D. Mediterranean diet and weight loss: meta-analysis of randomized controlled trials. Metab Syndr Relat Disord. 2011;9(1):1-12.
69. Katz DL MS. Can we say what diet is best for health? Annu Rev Public Health. 2014;35:83-103.
70. Martinez-Gonzalez MA, Gea A, Ruiz-Canela M. The Mediterranean Diet and Cardiovascular Health. Circ Res. 2019;124(5):779-98.

71. Hu EA, Toledo E, Diez-Espino J, Estruch R, Corella D, Salas-Salvado J, et al. Lifestyles and risk factors associated with adherence to the Mediterranean diet: a baseline assessment of the PREDIMED trial. PLoS One. 2013;8(4):e60166.
72. Helsedirektoratet. Anbefalinger om kosthold, ernæring og fysisk aktivitet. Sosial- og helsedirektoratet. 2014.
73. Kromhout D, Yasuda S, Geleijnse JM, Shimokawa H. Fish oil and omega-3 fatty acids in cardiovascular disease: do they really work? Eur Heart J. 2012;33(4):436-43.
74. Jacobson TA. Role of n-3 fatty acids in the treatment of hypertriglyceridemia and cardiovascular disease. Am J Clin Nutr. 2008;87(6):1981S-90S.
75. Kris-Etherton PM, Harris WS, Appel LJ, American Heart Association. Nutrition C. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation. 2002;106(21):2747-57.
76. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet. 1989;2(8666):757-61.
77. Karimi G, Heidari Z, Firouzi S, Haghighatdoost F. A systematic review and meta-analysis of the association between fish consumption and risk of metabolic syndrome. Nutr Metab Cardiovasc Dis. 2020;30(5):717-29.
78. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Marine n-3 Fatty Acids and Prevention of Cardiovascular Disease and Cancer. N Engl J Med. 2019;380(1):23-32.
79. Kwak SM, Myung SK, Lee YJ, Seo HG, Korean Meta-analysis Study G. Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular disease: a meta-analysis of randomized, double-blind, placebo-controlled trials. Arch Intern Med. 2012;172(9):686-94.
80. https://ods.od.nih.gov/factsheets/Omega3FattyAcids-HealthProfessional/.
81. Kris-Etherton PM, Richter CK, Bowen KJ, Skulas-Ray AC, Jackson KH, Petersen KS, et al. Recent Clinical Trials Shed New Light on the Cardiovascular Benefits of Omega-3 Fatty Acids. Methodist Debakey Cardiovasc J. 2019;15(3):171-8.
82. Hu Y, Hu FB, Manson JE. Marine Omega-3 Supplementation and Cardiovascular Disease: An Updated Meta-Analysis of 13 Randomized Controlled Trials Involving 127 477 Participants. J Am Heart Assoc. 2019;8(19):e013543.
83. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. N Engl J Med. 2019;380(1):11-22.
84. Zhang Y, Zhuang P, He W, Chen JN, Wang WQ, Freedman ND, et al. Association of fish and long-chain omega-3 fatty acids intakes with total and cause-specific mortality: prospective analysis of 421 309 individuals. J Intern Med. 2018;284(4):399-417.
85. Grosso G, Yang J, Marventano S, Micek A, Galvano F, Kales SN. Nut consumption on all-cause, cardiovascular, and cancer mortality risk: a systematic review and meta-analysis of epidemiologic studies. Am J Clin Nutr. 2015;101(4):783-93.
86. Tan SY, Dhillon J, Mattes RD. A review of the effects of nuts on appetite, food intake, metabolism, and body weight. Am J Clin Nutr. 2014;100 Suppl 1:412S-22S.
87. Guasch-Ferre M, Liu X, Malik VS, Sun Q, Willett WC, Manson JE, et al. Nut Consumption and Risk of Cardiovascular Disease. J Am Coll Cardiol. 2017;70(20):2519-32.
88. Kim Y, Keogh J, Clifton PM. Nuts and Cardio-Metabolic Disease: A Review of Meta-Analyses. Nutrients. 2018;10(12).
89. Batterman SA, Chernyak S, Su FC. Measurement and Comparison of Organic Compound Concentrations in Plasma, Whole Blood, and Dried Blood Spot Samples. Front Genet. 2016;7:64.
90. De Roos AJ, Ulrich CM, Sjodin A, McTiernan A. Adiposity, body composition, and weight change in relation to organochlorine pollutant plasma concentrations. J Expo Sci Environ Epidemiol. 2012;22(6):617-24.
91. Berg V, Nost TH, Sandanger TM, Rylander C. Predicting human plasma concentrations of persistent organic pollutants from dietary intake and socio-demographic information in the Norwegian Women and Cancer study. Environ Int. 2018;121(Pt 2):1311-8.
92. https://www.who.int/foodsafety/areas_work/chemical-risks/pops/en/.

93. Lind PM, Salihovic S, Stubleski J, Karrman A, Lind L. Association of Exposure to Persistent Organic Pollutants With Mortality Risk: An Analysis of Data From the Prospective Investigation of Vasculature in Uppsala Seniors (PIVUS) Study. JAMA Netw Open. 2019;2(4):e193070.
94. Fry K, Power MC. Persistent organic pollutants and mortality in the United States, NHANES 1999-2011. Environ Health. 2017;16(1):105.
95. Lyche JL, Nourizadeh-Lillabadi R, Karlsson C, Stavik B, Berg V, Skare JU, et al. Natural mixtures of POPs affected body weight gain and induced transcription of genes involved in weight regulation and insulin signaling. Aquat Toxicol. 2011;102(3-4):197-204.
96. Lind PM, Lee DH, Jacobs DR, Salihovic S, van Bavel B, Wolff MS, et al. Circulating levels of persistent organic pollutants are related to retrospective assessment of life-time weight change. Chemosphere. 2013;90(3):998-1004.
97. Kim MJ, Marchand P, Henegar C, Antignac JP, Alili R, Poitou C, et al. Fate and complex pathogenic effects of dioxins and polychlorinated biphenyls in obese subjects before and after drastic weight loss. Environ Health Perspect. 2011;119(3):377-83.
98. Hong NS, Kim KS, Lee IK, Lind PM, Lind L, Jacobs DR, et al. The association between obesity and mortality in the elderly differs by serum concentrations of persistent organic pollutants: a possible explanation for the obesity paradox. Int J Obes (Lond). 2012;36(9):1170-5.
99. Gasull M, Castell C, Pallares N, Miret C, Pumarega J, Te Llez-Plaza M, et al. Blood Concentrations of Persistent Organic Pollutants and Unhealthy Metabolic Phenotypes in Normal-Weight, Overweight, and Obese Individuals. Am J Epidemiol. 2018;187(3):494-506.
100. Henriquez-Hernandez LA, Luzardo OP, Zumbado M, Serra-Majem L, Valeron PF, Camacho M, et al. Determinants of increasing serum POPs in a population at high risk for cardiovascular disease. Results from the PREDIMED-CANARIAS study. Environ Res. 2017;156:477-84.
101. Berg ZK, Rodriguez B, Davis J, Katz AR, Cooney RV, Masaki K. Association Between Occupational Exposure to Pesticides and Cardiovascular Disease Incidence: The Kuakini Honolulu Heart Program. J Am Heart Assoc. 2019;8(19):e012569.
102. Gauthier MS, Rabasa-Lhoret R, Prud'homme D, Karelis AD, Geng D, van Bavel B, et al. The metabolically healthy but obese phenotype is associated with lower plasma levels of persistent organic pollutants as compared to the metabolically abnormal obese phenotype. J Clin Endocrinol Metab. 2014;99(6):E1061-6.
103. Taylor KW, Novak RF, Anderson HA, Birnbaum LS, Blystone C, Devito M, et al. Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: a national toxicology program workshop review. Environ Health Perspect. 2013;121(7):774-83.
104. Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs DR, Jr. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. PLoS One. 2011;6(1):e15977.
105. Wu H, Bertrand KA, Choi AL, Hu FB, Laden F, Grandjean P, et al. Persistent organic pollutants and type 2 diabetes: a prospective analysis in the nurses' health study and meta-analysis. Environ Health Perspect. 2013;121(2):153-61.
106. https://www.food.gov.uk/business-guidance/persistent-organic-pollutants. 2019.
107. Myrmel LS, Fjaere E, Midtbo LK, Bernhard A, Petersen RK, Sonne SB, et al. Macronutrient composition determines accumulation of persistent organic pollutants from dietary exposure in adipose tissue of mice. J Nutr Biochem. 2016;27:307-16.
108. Lee YM, Kim KS, Jacobs DR, Jr., Lee DH. Persistent organic pollutants in adipose tissue should be considered in obesity research. Obes Rev. 2017;18(2):129-39.
109. Hites RA, Foran JA, Carpenter DO, Hamilton MC, Knuth BA, Schwager SJ. Global assessment of organic contaminants in farmed salmon. Science. 2004;303(5655):226-9.
110. Raising efficiency sisf. Raising efficiency, sustainability in salmon farming. https://physorg/news/2014-01-efficiency-sustainability-salmon-farminghtml. 2014.
111. Lundebye AK, Lock EJ, Rasinger JD, Nostbakken OJ, Hannisdal R, Karlsbakk E, et al. Lower levels of Persistent Organic Pollutants, metals and the marine omega 3-fatty acid DHA in farmed compared to wild Atlantic salmon (Salmo salar). Environ Res. 2017;155:49-59.

112. Wangensteen T, Kolsgaard ML, Mattingsdal M, Joner G, Tonstad S, Undlien D, et al. Mutations in the melanocortin 4 receptor (MC4R) gene in obese patients in Norway. Exp Clin Endocrinol Diabetes. 2009;117(6):266-73.
113. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004;27(6):1487-95.
114. Koponen J, Rantakokko P, Airaksinen R, Kiviranta H. Determination of selected perfluorinated alkyl acids and persistent organic pollutants from a small volume human serum sample relevant for epidemiological studies. J Chromatogr A. 2013;1309:48-55.
115. Sabrina Nour GP. Pharmacoepidemiology in the Prevention of Adverse Drug Reactions. Pharmacoepidemiology and Pharmacovigilance. 2019;3.6.1 Selection Bias.
116. Artama M, Heinavaara S, Sarkeala T, Prattala R, Pukkala E, Malila N. Determinants of non-participation in a mass screening program for colorectal cancer in Finland. Acta Oncol. 2016;55(7):870-4.
117. He E, Lew JB, Egger S, Banks E, Ward RL, Beral V, et al. Factors associated with participation in colorectal cancer screening in Australia: Results from the 45 and Up Study cohort. Prev Med. 2018;106:185-93.
118. Connor Gorber S, Schofield-Hurwitz S, Hardt J, Levasseur G, Tremblay M. The accuracy of self-reported smoking: a systematic review of the relationship between self-reported and cotinine-assessed smoking status. Nicotine Tob Res. 2009;11(1):12-24.
119. Simundic AM. Bias in research. Biochem Med (Zagreb). 2013;23(1):12-5.
120. Coughlin SS. Recall bias in epidemiologic studies. J Clin Epidemiol. 1990;43(1):87-91.
121. Dahm CC. Correcting measurement error in dietary exposure assessments: no piece of cake. Am J Clin Nutr. 2020.
122. Skelly AC, Dettori JR, Brodt ED. Assessing bias: the importance of considering confounding. Evid Based Spine Care J. 2012;3(1):9-12.
123. Khorsan R, Crawford C. How to assess the external validity and model validity of therapeutic trials: a conceptual approach to systematic review methodology. Evid Based Complement Alternat Med. 2014;2014:694804.
124. http://www.indiana.edu/~p1013447/dictionary/int_val.htm.
125. Katz R. Biomarkers and surrogate markers: an FDA perspective. NeuroRx. 2004;1(2):189-95.
126. Collaboration CRPCHDG, Wensley F, Gao P, Burgess S, Kaptoge S, Di Angelantonio E, et al. Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. BMJ. 2011;342:d548.
127. Weintraub WS, Luscher TF, Pocock S. The perils of surrogate endpoints. Eur Heart J. 2015;36(33):2212-8.
128. Kim ST, Kim BJ, Lim DM, Song IG, Jung JH, Lee KW, et al. Basal C-peptide Level as a Surrogate Marker of Subclinical Atherosclerosis in Type 2 Diabetic Patients. Diabetes Metab J. 2011;35(1):41-9.
129. Leighton E, Sainsbury CA, Jones GC. A Practical Review of C-Peptide Testing in Diabetes. Diabetes Ther. 2017;8(3):475-87.
130. Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med. 2013;30(7):803-17.
131. Kramer CK, Zinman B, Retnakaran R. Are metabolically healthy overweight and obesity benign conditions?: A systematic review and meta-analysis. Ann Intern Med. 2013;159(11):758-69.
132. Park JT, Chang TI, Kim DK, Choi HY, Lee JE, Kim HW, et al. Association of white blood cell count with metabolic syndrome in patients undergoing peritoneal dialysis. Metabolism. 2009;58(10):1379-85.
133. Faria G, Goncalves A, Cunha R, Guimaraes JT, Calhau C, Preto J, et al. Beyond central adiposity: liver fat and visceral fat area are associated with metabolic syndrome in morbidly obese patients. Int J Surg. 2015;14:75-9.
134. Robberecht H, Hermans N. Biomarkers of Metabolic Syndrome: Biochemical Background and Clinical Significance. Metab Syndr Relat Disord. 2016;14(2):47-93.
135. Lim Y, Yoo S, Lee SA, Chin SO, Heo D, Moon JC, et al. Apolipoprotein B Is Related to Metabolic Syndrome Independently of Low Density Lipoprotein Cholesterol in Patients with Type 2 Diabetes. Endocrinol Metab (Seoul). 2015;30(2):208-15.

136. Majid H, Masood Q, Khan AH. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR): A Better Marker for Evaluating Insulin Resistance Than Fasting Insulin in Women with Polycystic Ovarian Syndrome. J Coll Physicians Surg Pak. 2017;27(3):123-6.
137. Turner RC, Holman RR, Matthews D, Hockaday TD, Peto J. Insulin deficiency and insulin resistance interaction in diabetes: estimation of their relative contribution by feedback analysis from basal plasma insulin and glucose concentrations. Metabolism. 1979;28(11):1086-96.
138. Okura T, Nakamura R, Fujioka Y, Kawamoto-Kitao S, Ito Y, Matsumoto K, et al. CPR-IR is an insulin resistance index that is minimally affected by hepatic insulin clearance-A preliminary research. PLoS One. 2018;13(5):e0197663.
139. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 1979;237(3):E214-23.
140. Kim JK. Hyperinsulinemic-euglycemic clamp to assess insulin sensitivity in vivo. Methods Mol Biol. 2009;560:221-38.
141. Ruzzin J, Petersen R, Meugnier E, Madsen L, Lock EJ, Lillefosse H, et al. Persistent organic pollutant exposure leads to insulin resistance syndrome. Environ Health Perspect. 2010;118(4):465-71.
142. Faerch K, Hojlund K, Vind BF, Vaag A, Dalgard C, Nielsen F, et al. Increased serum concentrations of persistent organic pollutants among prediabetic individuals: potential role of altered substrate oxidation patterns. J Clin Endocrinol Metab. 2012;97(9):E1705-13.
143. Ngwa EN, Kengne AP, Tiedeu-Atogho B, Mofo-Mato EP, Sobngwi E. Persistent organic pollutants as risk factors for type 2 diabetes. Diabetol Metab Syndr. 2015;7:41.
144. Porta M, Pumarega J, Gasull M, Lopez T. Contamination from endocrine disrupters of the general population at low and high concentrations. Vitam Horm. 2014;94:167-92.
145. Ukkola OH, Puurunen VP, Piira OP, Niva JT, Lepojarvi ES, Tulppo MP, et al. High serum fasting peptide YY (3-36) is associated with obesity-associated insulin resistance and type 2 diabetes. Regul Pept. 2011;170(1-3):38-42.
146. Fernandez-Garcia JC, Murri M, Coin-Araguez L, Alcaide J, El Bekay R, Tinahones FJ. GLP-1 and peptide YY secretory response after fat load is impaired by insulin resistance, impaired fasting glucose and type 2 diabetes in morbidly obese subjects. Clin Endocrinol (Oxf). 2014;80(5):671-6.
147. Smith RM, Klein R, Kruzliak P, Zulli A. Role of Peptide YY in blood vessel function and atherosclerosis in a rabbit model. Clin Exp Pharmacol Physiol. 2015;42(6):648-52.
148. Adrian TE, Sagor GR, Savage AP, Bacarese-Hamilton AJ, Hall GM, Bloom SR. Peptide YY kinetics and effects on blood pressure and circulating pancreatic and gastrointestinal hormones and metabolites in man. J Clin Endocrinol Metab. 1986;63(4):803-7.
149. Sima E, Webb DL, Hellstrom PM, Sundbom M. Non-responders After Gastric Bypass Surgery for Morbid Obesity: Peptide Hormones and Glucose Homeostasis. Obes Surg. 2019.
150. Weise CM, Thiyyagura P, Reiman EM, Chen K, Krakoff J. Postprandial plasma PYY concentrations are associated with increased regional gray matter volume and rCBF declines in caudate nuclei--a combined MRI and H2(15)O PET study. Neuroimage. 2012;60(1):592-600.
151. Ledoux S, Coupaye M, Essig M, Msika S, Roy C, Queguiner I, et al. Traditional anthropometric parameters still predict metabolic disorders in women with severe obesity. Obesity (Silver Spring). 2010;18(5):1026-32.
152. Zazai R, Wilms B, Ernst B, Thurnheer M, Schultes B. Waist circumference and related anthropometric indices are associated with metabolic traits in severely obese subjects. Obes Surg. 2014;24(5):777-82.
153. Ebron K, Andersen CJ, Aguilar D, Blesso CN, Barona J, Dugan CE, et al. A Larger Body Mass Index is Associated with Increased Atherogenic Dyslipidemia, Insulin Resistance, and Low-Grade Inflammation in Individuals with Metabolic Syndrome. Metab Syndr Relat Disord. 2015;13(10):458-64.
154. Plourde G, Karelis AD. Current issues in the identification and treatment of metabolically healthy but obese individuals. Nutr Metab Cardiovasc Dis. 2014;24(5):455-9.
155. Goncalves CG, Glade MJ, Meguid MM. Metabolically healthy obese individuals: Key protective factors. Nutrition. 2016;32(1):14-20.
156. Klop B, Elte JW, Cabezas MC. Dyslipidemia in obesity: mechanisms and potential targets. Nutrients. 2013;5(4):1218-40.

157. Tinahones FJ, Garrido-Sanchez L, Miranda M, Garcia-Almeida JM, Macias-Gonzalez M, Ceperuelo V, et al. Obesity and insulin resistance-related changes in the expression of lipogenic and lipolytic genes in morbidly obese subjects. Obes Surg. 2010;20(11):1559-67.
158. Lind L, Salihovic S, Lampa E, Lind PM. Mixture effects of 30 environmental contaminants on incident metabolic syndrome-A prospective study. Environ Int. 2017;107:8-15.
159. Arrebola JP, Fernandez MF, Martin-Olmedo P, Bonde JP, Martin-Rodriguez JL, Exposito J, et al. Historical exposure to persistent organic pollutants and risk of incident hypertension. Environ Res. 2015;138:217-23.
160. Goncharov A, Pavuk M, Foushee HR, Carpenter DO, Anniston Environmental Health Reseach C. Blood pressure in relation to concentrations of PCB congeners and chlorinated pesticides. Environ Health Perspect. 2011;119(3):319-25.
161. Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR, Jr. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetologia. 2007;50(9):1841-51.
162. Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetes Care. 2007;30(3):622-8.
163. Wolff MS, Zeleniuch-Jacquotte A, Dubin N, Toniolo P. Risk of breast cancer and organochlorine exposure. Cancer Epidemiol Biomarkers Prev. 2000;9(3):271-7.
164. Kim KS, Hong NS, Jacobs DR, Jr., Lee DH. Interaction Between Persistent Organic Pollutants and C-reactive Protein in Estimating Insulin Resistance Among Non-diabetic Adults. J Prev Med Public Health. 2012;45(2):62-9.
165. Gauthier M-S R-LR, Prud'homme D, Karelis AD, Geng D, van Bavel B, Ruzzin J. Author Response to Ayotte et al, The metabolically healthy but obese phenotype is associated with lower plasma levels of persistent organic pollutants as compared to the metabolically abnormal obese phenotype. http://pressendocrineorg/e-letters/101210/jc2013-3935 2014.
166. Pestana D, Faria G, Sa C, Fernandes VC, Teixeira D, Norberto S, et al. Persistent organic pollutant levels in human visceral and subcutaneous adipose tissue in obese individuals--depot differences and dysmetabolism implications. Environ Res. 2014;133:170-7.
167. Kim KS, Lee YM, Kim SG, Lee IK, Lee HJ, Kim JH, et al. Associations of organochlorine pesticides and polychlorinated biphenyls in visceral vs. subcutaneous adipose tissue with type 2 diabetes and insulin resistance. Chemosphere. 2014;94:151-7.
168. Tani S, Matsuo R, Imatake K, Suzuki Y, Takahashi A, Matsumoto N. Association of daily fish intake with serum non-high-density lipoprotein cholesterol levels and healthy lifestyle behaviours in apparently healthy males over the age of 50 years in Japanese: Implication for the anti-atherosclerotic effect of fish consumption. Nutr Metab Cardiovasc Dis. 2020;30(2):190-200.
169. Shi H, Jan J, Hardesty JE, Falkner KC, Prough RA, Balamurugan AN, et al. Polychlorinated biphenyl exposures differentially regulate hepatic metabolism and pancreatic function: Implications for nonalcoholic steatohepatitis and diabetes. Toxicol Appl Pharmacol. 2019;363:22-33.
170. Dusanov S, Ruzzin J, Kiviranta H, Klemsdal TO, Retterstol L, Rantakokko P, et al. Associations between persistent organic pollutants and metabolic syndrome in morbidly obese individuals. Nutr Metab Cardiovasc Dis. 2018;28(7):735-42.
171. Vassilopoulou L, Psycharakis C, Petrakis D, Tsiaoussis J, Tsatsakis AM. Obesity, Persistent Organic Pollutants and Related Health Problems. Adv Exp Med Biol. 2017;960:81-110.
172. Jorgensen ME, Borch-Johnsen K, Bjerregaard P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance among Greenland Inuit. Diabetologia. 2008;51(8):1416-22.
173. Henriquez-Hernandez LA, Luzardo OP, Valeron PF, Zumbado M, Serra-Majem L, Camacho M, et al. Persistent organic pollutants and risk of diabetes and obesity on healthy adults: Results from a cross-sectional study in Spain. Sci Total Environ. 2017;607-608:1096-102.
174. Manninen SM, Lankinen MA, de Mello VD, Laaksonen DE, Schwab US, Erkkila AT. Intake of Fatty Fish Alters the Size and the Concentration of Lipid Components of HDL Particles and Camelina Sativa Oil Decreases IDL Particle Concentration in Subjects with Impaired Glucose Metabolism. Mol Nutr Food Res. 2018;62(10):e1701042.

175. Marushka L, Batal M, David W, Schwartz H, Ing A, Fediuk K, et al. Association between fish consumption, dietary omega-3 fatty acids and persistent organic pollutants intake, and type 2 diabetes in 18 First Nations in Ontario, Canada. Environ Res. 2017;156:725-37.
176. Bitok E, Sabate J. Nuts and Cardiovascular Disease. Prog Cardiovasc Dis. 2018;61(1):33-7.
177. Rajaram S, Haddad EH, Mejia A, Sabate J. Walnuts and fatty fish influence different serum lipid fractions in normal to mildly hyperlipidemic individuals: a randomized controlled study. Am J Clin Nutr. 2009;89(5):1657S-63S.
178. Kahleova H, Tonstad S, Rosmus J, Fisar P, Mari A, Hill M, et al. The effect of a vegetarian versus conventional hypocaloric diet on serum concentrations of persistent organic pollutants in patients with type 2 diabetes. Nutr Metab Cardiovasc Dis. 2016;26(5):430-8.
179. Bou M, Berge GM, Baeverfjord G, Sigholt T, Ostbye TK, Ruyter B. Low levels of very-long-chain n-3 PUFA in Atlantic salmon (Salmo salar) diet reduce fish robustness under challenging conditions in sea cages. J Nutr Sci. 2017;6:e32.
180. Li H, Zhu D, Lu X, Du H, Guan S, Chen Z. Determination and risk assessment of sixteen polycyclic aromatic hydrocarbons in vegetables. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2018;53(2):116-23.
181. Hausken T, Skaare JU, Polder A, Haugen M, Meltzer HM, Lundebye AK, et al. High consumption of farmed salmon does not disrupt the steady state of persistent organic pollutants (POP) in human plasma and adipose tissue. J Toxicol Environ Health A. 2014;77(20):1229-50.
182. Lee YM, Lee DH. Letter to the Editor: Effect of fatty fish or nut consumption on concentrations of persistent organic pollutants in overweight or obese men and women: A randomized controlled clinical trial. Nutr Metab Cardiovasc Dis. 2020.
183. Tonstad SK, TO. Dusanov, S, Gulseth H. https://www.nmcd-journal.com/article/S0939-4753(20)30067-3/pdf. NMCD. 2020.

13. Appendix

Sjekkliste for deltakere i studien

OPPFYLLER FØLGENDE KRITERIER FOR METABOLSK

SYNDROM:SCREENINGNUMMER:

Kjønn: mann kvinne

Livvidde: ≥102/88 cm	ja	nei
Triglyserider: ≥ 1,7	ja	nei
HDL-kolesterol ≤ 1,0/1,3	ja	nei
Blodtrykk ≥ 130/85 eller bruk av medisin	ja	nei
Blodsukker ≥ 5,6	ja	nei

Det ble tatt utskrift av blodprøvesvaret	ja	nei

Dato og signatur lege ansvarlig for screening _____

Er du overvektig og interessert i kostholdet?

Ved Seksjon for preventiv kardiologi, Oslo Universitetssykehus, vil vi undersøke helseeffektene av å spise fet fisk eller nøtter.

Du kan delta i studien hvis du:

- Bor i Oslo eller omegn, eller er ofte i Oslo?
- Er i alder 40-65 år?

Har overvekt eller moderat fedme med BMI (kroppsmasseindeks) 25-35 kg/m^2

Du kan ikke delta dersom du: Røyker, har diabetes type 1 eller 2, er allergisk overfor fet fisk eller nøtter.

I studien vil du bli tilfeldig trukket ut til å være i 1 av 3 grupper:

- Fiskegruppen som skal spise fet fisk og unngå nøtter (kan i tillegg spise mager fisk eller sjømat)
- Nøttegruppen som skal spise nøtter og unngå fet fisk (men kan spise mager fisk eller sjømat)
- Kontrollgruppen som skal unngå både nøtter og fet fisk (men kan spise mager fisk eller sjømat)

All oppfølging under studien og studiematen er gratis.

Kontroll gruppe får et gavekort på **1200 kr**.

- Studien krever at du møter opp i alt ca. 6-10 ganger i løpet av 1/2 år ved Seksjon for preventiv kardiologi, Oslo Universitetssykehus, Ullevål til undersøkelse og utlevering av matvarene. Vi tar hensyn til dine andre planer når vi setter opp avtalene.

Oppfølging hos klinisk ernæringsfysiolog rett etter din deltakelse for eventuell vektreduksjon tilbys til alle interesserte.

Hvis du er interessert i å delta eller ønsker mer informasjon om prosjektet ta uforpliktende kontakt helst på e-post sasdus@ous-hf.no eller alternativt på telefon

22118619 eller mobil 46425054.

MÅLING AV INSULINFØLSOMHET I FORSKNINGSPROSJEKTET «ORGANISKE MILJØGIFTER OG FEDME»

Protocol clamp:

Background:

All subjects will have their insulin sensitivity measured from fasting surrogate markers. In a subsample estimating insulin sensitivity with state of the art methodology is highly warranted.

Aim: To estimate insulin sensitivity measured as glucose infusion rate pre and post-intervention.

Measurement of insulin action by the use of euglycemic hyperinsulinemic clamp

Euglycemic hyperinsulinemic clamp

An initial 9 minutes priming infusion, determined by the participant's pre-clamp plasma glucose, is given. Insulin 300 mU x ml^{-1} is then infused at a rate of 40 mU x m^{-2} x min^{-1}. Plasma glucose is measured every 5 minutes. When plasma glucose is less than 6.0 mmol x l^{-1} a variable infusion of glucose 200 mg x ml^{-1} is begun, and subsequently adjusted to obtain plasma glucose close to 5 mmol x l^{-1}. Total clamp duration is 150 minutes. During the last 30 minutes of the clamp, serum insulin are measured. The glucose infusion rate is calculated from the last 30 minutes of stable euglycemia, and is expressed in µmol x m^{-2} x min^{-1}. The euglycemic hyperinsulinemic clamp will be performed at the Diabetes Research Laboratory at Oslo University Hospital Aker headed by Dr Hanne L Gulseth. The clamp will be performed for 15 subjects in the control and 15 patients in the fish group pre and post-intervention.

Forespørsel om deltakelse i forskningsprosjektet

MÅLING AV INSULINFØLSOMHET I FORSKNINGSPROSJEKTET «ORGANISKE MILJØGIFTER OG FEDME»

Dette er et spørsmål til deg om å delta i en understudie av forskningsprosjekt «Miljøgifterog fedme» å se om det er forskjell i insulinfølsomhet etter inntak av fisk eller nøtter.

Hva innebærer PROSJEKTET?

I tillegg til din ordinære deltakelse i prosjektet «Organiske miljøgifterog fedme» vil vi i dette underprosjektet måle insulinfølsomheten din ved hjelp av gullstandardmetoden for ca 15 pasienter i hver intervensjonsgruppe.

Måling av insulinfølsomhet gjøres ved hjelp av en metode kalt euglykemisk hyperinsulinemisk clamp - dette er en undersøkelse hvor du møter fastende og vi gir deg insulin og sukkervann gjennom en kanyle i den ene albuen, og måler blodsukker jevnlig via en kanyle i den andre albuen. Undersøkelsen varer i 3-4 timer.

Mulige fordeler og ulemper

Fordelen ved å delta er at du får kartlagt om du er insulinfølsom eller insulinresistent. Dette er en relativt kostbar og komplisert undersøkelse og gjøres derfor sjelden rutinemessig. Ulempen ved å delta er at det tar tid, ca 4 timer før og 4 timer, etter intervensjonsstudien. undersøkelser. Undersøkelsene du skal gjennom medfører at det blir tatt blodprøver, og dersom vi ønsker å ta gjentatte prøver samme dag vil vi legge inn kanyler (plastrør) i armene så du slipper å bli stukket mer enn en gang i hver arm. Ved clampundersøkelsen vil du få drypp med sukkervann som i noen tilfeller kan virke irriterende på blodåren. Dette forsøker vi å unngå ved å ikke gi for høye doser. Dersom du skulle oppleve svie armen under undersøkelsen vil vi gi ekstra saltvann for å dempe irritasjonen, og du vil få utdelt en lindrende salve etter undersøkelsen. Ubehaget forsvinner etter noen timer eller et par dager, og gir ikke varig mén.

Frivillig deltakelse og mulighet for å trekke sitt samtykke

Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Dersom du trekker deg fra prosjektet, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte dr Sasa Dusanov tlf. 98056904

Hva skjer med informasjonen om deg?

Informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert. Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger gjennom en navneliste.

Forsikring

Pasientskadeloven gjelder for deltakere i medisinske forskningsprosjekter på sykehusene på lik linje med ordinære sykehuspasienter. Ved eventuell skade vil deltakerne kunne kreve erstatning fra Norsk Pasientskadeerstatning.

Utlevering av opplysninger til andre

Ved å delta i prosjektet, samtykker du også til at opplysninger om insulinfølsomheten din kan utleveres til utlandet sammen med andre opplysninger som beskrevet i hovedprosjektet. Dette kan være land med lover som ikke tilfredsstiller europeisk personvernlovgivning. Koden som knytter deg til dine personidentifiserende opplysninger vil ikke bli utlevert.

Godkjenning

Prosjektet er godkjent av Regional komite for medisinsk og helsefaglig forskningsetikk, (2015/1930-8)

Samtykke til deltakelse i PROSJEKTET

Jeg er villig til å delta

Sted og dato	Deltakers signatur
	Deltakers navn med trykte bokstaver

Jeg bekrefter å ha gitt informasjon om prosjektet

Sted og dato	Signatur
	Rolle i prosjektet

Telefonintervju Fiskeprosjektet

Intervju:

Navn:

Fødselsdato:

Alder:

Kjønn:

Adresse:

E-post:

Telefon:

Mobil:

Høyde: Vekt: KMI (kg/m^2):

Stabil vekt siste året (mindre enn 3-5 kg endring)	ja	nei
Gjennomført kirurgisk behandling for vektreduksjon	ja	nei
Pågående bruk av medikamenter eller urter for vektreduksjon	ja	nei
Bruk av kosttilskudd	ja	nei
Diabetes type I eller type II behandlet eller ubehandlet	ja	nei
Spiseforstyrrelser	ja	nei
Psykisk sykdom som gir utfordringer i forhold til studieprosedyrene	ja	nei
Alkohol, stoff- eller pillemisbruk, tidligere eller nå	ja	nei
Sykdom inkludert gastrointestinal sykdom og allergi som påvirker compliance	ja	nei

Gravid, ammende eller planlagt graviditet i studieperioden	ja	nei
Alvorlig sykdom som er en kontraindikasjon for å delta	ja	nei
Allergi mot fisk, sjømat eller nøtter (mandler, valnøtter, hasselnøtter)	ja	nei
Villig til å møte opp 10 ganger iløpet av 6 måneders periode	ja	nei
Villig til å registrere kostholdet og følge kostplanen avtalt med studieleder	ja	nei
Du godtar at dette ikke er en vektreduksjonsstudie	ja	nei
Du er villig til å bli randomisert til 1 av 3 grupper (fisk, nøtt, kontroll)	ja	nei
Du ønsker å delta i en klinisk studie frivillig	ja	nei
Er det OK for deg å møte fastende enkelte ganger for blodprøver	ja	nei
Planlegger du en lengre ferie	ja	nei

Forespørsel om deltakelse

Fisk eller nøtter? Kostholdseffektene på kardiometabolske risikofaktorer og persisterende miljøgifter

Bakgrunn og hensikt

Dette er en forespørsel til deg om å delta i en forskningsstudie som undersøker effekten av fet fisk eller nøtter i forhold til metabolske risikofaktorer. Hensikten med studien er å undersøke mulige antatte positive effekter av fet fisk eller nøtter og å kartlegge eventuelle negative effekter av miljøgifter. Det betyr at vi vil spesifikt undersøke effekten av de nevnte matvarene på ulike risikofaktorer for hjerte- og karsykdom. Du blir spurt om å delta i studien fordi du er overvektig og har økt risiko for å få hjerte- og karsykdom. Miljøgifter fra fet fisk har fått mye oppmerksomhet i media, men er lite undersøkt som årsak til metabolske forstyrrelser som diabetes eller overvekt/fedme. Studien vil kunne bidra til presisering av kostråd for personer med de metabolske forstyrrelsene og overvekt/fedme. Studien finansieres av Extrastiftelsen via Nasjonalforeningen for folkehelsen og Oslo universitetssykehus.

Hva innebærer studien?

Totalt 120 forsøkspersoner i Oslo og omegn vil delta i studien ved Seksjon for preventiv kardiologi, Oslo universitetssykehus. Hvis det etter første besøk viser seg at du er aktuell for deltakelse i studien vil du bli tilfeldig valgt ut til **én** av følgende grupper:

Gruppe 1: Du blir bedt om å unngå all fet fisk eller nøtter de første 2 ukene slik at alle deltagerne får en lik start. Etter dette vil du bli randomisert til en av 3 grupper. Du vil få personlige kostråd for hvordan du kan spise fet fisk minst 3-4 ganger per uke. Fisken du skal spise vil du få gratis av oss i 6 måneder og du kan komme og hente den etter skjema du finner nederst i dette skrivet. Du vil få frosset laksefilet og makrell i tomatsaus slik at fisken kan spises som middag eller lunsj. Du vil få utdelt 500 g laks og 1 boks makrell i tomatsaus hver uke. Dette tilsvarer rundt 1400 kcal per uke. Du må unngå nøtter hvis du er i denne

fiskegruppen. Du vil få oppfølging hos lege og ernæringsfysiolog - vi kan svarer deg på alle spørsmål du eventuelt måtte ha. Fysisk aktivitet skal være uforandret i perioden du deltar i studiene.

Gruppe 2: Du blir bedt om å unngå all fet fisk eller nøtter de første 2 ukene slik at alle deltagerne får en lik start. Etter dette blir du randomisert til en av 3 grupper. Hvis du havner i gruppe 2 skal du spise ca 200g nøtter per uke fordelt på 100 g valnøtter, 50 g hasselnøtter og 50 g mandler. Dette tilsvarer omtrent 1400 kcal per uke. Du vil få personlige kostråd for hvordan du kan spise denne mengden nøtter uten å gå opp i vekt. Nøttene får du gratis av oss i 6 måneder. Nøtter kan du spise til eller mellom måltidene. Du må unngå fet fisk hvis du er i nøttegruppen. Du vil få oppfølging hos lege og ernæringsfysiolog og vi vil svare deg på alle spørsmål du eventuelt måtte ha.. Fysisk aktivitet skal være uforandret i perioden du deltar i studiene.

Gruppe 3: Du blir bedt om å unngå all fet fisk eller nøtter de første 2 ukene slik at alle deltagerne får en lik start. Etter dette skal du bli randomisert til en av 3 grupper. I gruppe 3 skal du unngå fet fisk eller nøtter i hele studieperioden. Du skal fortsette å spise slik du alltid har gjort. Etter du har fullført prosjektet vil du få et gavekort på 1200 kroner som kan brukes til å kjøpe fet fisk eller nøtter. Du vil få oppfølging hos lege og ernæringsfysiolog og vi vil svare deg på alle spørsmål du eventuelt måtte ha. Fysisk aktivitet skal være uforandret i perioden du deltar i studiene.

Studien vil innebære 10 visitter ved avdelingen i løpet av 1/2 år. Konsultasjonene vil vare fra 1 til 1,5 timer hver gang. De visittene vil innebære samtaler med lege og ernæringsfysiolog, henting av matvarer, legeundersøkelser og måling av blodtrykk, puls, vekt, livvidde og

fastende blodprøver. En mer detaljert oversikt over hvilke undersøkelser, prøver og aktiviteter som skjer på hvert besøk finner du på skjema nederst i dette skrivet.

Hvis du ønsker, kan du bestemme deg for å avslutte studien når som helst underveis og målingene som ble gjort før dette ikke skal brukes i studien uten ditt samtykke.

Studiedeltakerens ansvar

Du må komme til avtalte studiebesøk og spise etter de kostrådene du får så godt du kan. På hvert besøk må du informere lege eller ernæringsfysiologen om eventuelle symptomer, sykdommer og ubehag du har hatt. Du må fortelle om eventuelle medisiner du har tatt, også kosttilskudd og vitaminer.

Du må møte fastende på 4 av totalt 10 besøk (dvs. ikke spise eller drikke på 10 timer, unntatt et lite glass vann, svart kaffe og vanlig (svart) te). Du kan ta dine eventuelle medisiner som vanlig selv om du møter fastende.

Mulige fordeler, ulemper og bivirkninger

Kostrådene og inntaket av de ulike matvarene i fisk- og nøttegrupper vil kunne føre til forskjellige effekter. Vi forventer ikke at du skal gå opp eller ned i vekt i denne perioden. Det er mulig at gruppen som spiser fet fisk får noe økt mengde miljøgifter etter denne perioden, og at dette kunne påvirke de nevnte metabolske risikofaktorene i løpet av den tiden. Dette vil kunne føre til mulige endringer i nasjonale kostanbefalinger når det gjelder fet fisk. Fet fisk kan også ha positive effekter på hjerte- og karsykdom. Nøtter vil kunne føre til gevinster når det gjelder reduksjon av risiko for hjerte- og karsykdom. Begge gruppene vil kunne gå opp i vekt, men man får regelmessig veiledning fra klinisk ernæringsfysiolog nettopp for å unngå vektøkning. Gruppen som verken spiser nøtter eller fet fisk vil kunne ha et fullverdig kosthold allikevel.

Alle prøver, undersøkelser og veiledning er gratis. Du vil få ekstra tett oppfølging av lege og ernæringsfysiolog gjennom faste studiebesøk. Oppfølgingen hos lege og ernæringsfysiologen vil kunne inspirere og motivere deg til å gjøre flere gunstige kostholdsendringer med positive helseeffekter. Reiseutgifter i forbindelse med studiebesøkene blir ikke dekket. Det kan hende at man oppdager nye risikofaktorer eller verdier som eventuelt burde behandles med medisiner. Du vil da få hjelp med dette og få resepter som er nødvendige. Medisiner må du da betale selv men ikke konsultasjonen for dette. Hvis det er behov for andre målinger kan det hende at du må betale dette selv. Blodprøver skal også testes for miljøgifter. Dette skal utføres i utlandet og alle prøver skal først få et ”løpenummer” (bli anonymisert) og deretter bli sendt til laboratoriet som finnes i Finland. Kun prosjektleder skal ha liste som forbinder ditt navn med løpenummeret. Denne skal makuleres etter at resultatene er bearbeidet.

Vi forventer at denne studien vil gi oss bedre forståelse av miljøgifter og deres rolle i metabolske forandringer i kroppen som igjen kan føre til overvekt/fedme, diabetes, hjerte og karsykdom

Hva skjer med prøvene og informasjonen om deg?
Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Listen som kan koble ditt navn til koden, vil kun bli oppbevart på sykehuset og bare personell med ansvar for studien har tilgang til denne. Alle registrerte opplysninger vil bli lagret i 10 år. Det vil ikke være mulig å identifisere deg når resultatene fra studien publiseres.

Frivillig deltakelse

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke deg fra studien uten at det får konsekvenser for din videre behandling. Du må undertegne samtykkeerklæringen dersom du ønsker å delta, og du vil få med deg en kopi hjem.

Dersom du senere ønsker å trekke deg fra studien, har spørsmål til studien eller om dine rettigheter, ta kontakt med: Overlege Serena Tonstad tlf. 22 11 79 39 eller lege Sasa Dusanov tlf. 980 56 904 (bruk sms, så blir du oppringt)

Kapittel A- utdypende forklaring om hva studien innebærer

Kriterier for å delta

For å kunne delta i studien må du være mellom 40 til 65 år og ha en kroppsmasseindeks mellom 25-29.9 kg/m^2 (overvekt) eller mellom 30-38 kg/m^2 (fedme grad 1). og hatt stabil vekt ±3 kg de siste 3 månedene. Du må i tillegg ha **minst 1 risikofaktor** for hjerte- og karsykdom:

1. Forhøyet blodsukker

2. Forhøyet nivå av fettstoffer (triglyserider) i blodet

3. Lavt nivå av det gode (HDL) kolesterolet
4. Blodtrykk lik eller over 130/85
5. Livvidde over >102 cm for menn eller >94 cm for kvinner.

Du kan ikke delta i studien dersom du har aktiv alvorlig psykisk sykdom eller stoff- eller alkoholavhengighet. Du kan heller ikke ha diabetes som behandles med medisiner og/eller med insulin, hatt kirurgisk behandling for overvekt eller bruke vektreduserende medikamenter eller kosttilskudd for vektreduksjon. Tarmsykdom eller allergi mot fisk eller nøtter slik at du ikke kan spise den maten du får utlevert i studien, vil også hindre deg fra å kunne delta.

Bakgrunnsinformasjon om studien

Det ble gjort forskningsstudier tidligere som viser god effekt av nøtter på metabolske endringer i kroppen, som er nevnt før. Imidlertid studier hvor man undersøkte effekt av miljøgifter som finnes i animalske produkter, spesielt fisk har skapt mye interesse og debatt både i media og blant forskerne. I den nye rapporten fra Helsedirektoratet påstås det at mengde miljøgifter i norsk oppdrettsfisk har gått betraktelig ned og at man kunne spise fisk minst 3 ganger i uken. Vi ønsker å se forskjellene i verdier av kardiometabolske parametre når man spiser kun fet fisk sammenlignet med gruppe som spiser kun nøtter og gruppe som ikke spiser noe av delene.

Mulige ubehag, bivirkninger og risiko

Endring av kostsammensetning kan medføre ubehag fra mage og tarm. Ubehaget er som regel lett og forbigående. Du er fri til å trekke deg fra studien når du måtte ønske.

Ubehag i forbindelse med studieprosedyrer

Blodprøven krever nålestikk og du kan oppleve følgende ubehag: Smerter, blåmerke, svimmelhet og noen få kan besvime.

Studiedeltagerens ansvar

Legene ved Seksjon for preventiv kardiologi vil avgjøre om du fyller kravene for å delta. Visse medisiner og medisinske tilstander kan utelukke deg. Du må komme til avtalte visitter og undersøkelser og møte opp til møtene som avtalt. Dersom en annen lege vil gi deg nye medisiner mens du deltar i studien, må du informere om at du deltar i en forskningsstudie. Vennligst kontakt studiepersonalet før du starter på ny medisin hvis mulig.

Informer studieansvarlig umiddelbart dersom du: Får en skade eller har symptomer eller plager. Det er viktig at du rapporterer alle symptomer og bivirkninger umiddelbart gjennom hele studien, uansett om du tror det skyldes deltagelsen eller ikke.

Avbrutt studiedeltagelse

Legen eller studieansvarlig kan stoppe din deltagelse i studien hvis:

1. Du ikke følger opp studieprotokollen som avtalt.
2. Du får en alvorlig sykdom.
3. Legen som fører studien mener at deltagelse i studien ikke er til ditt beste.
4. Du blir gravid, planlegger å bli gravid eller ammer under studieperioden.

Informasjon til kvinner

Graviditet og amming vil kunne påvirke de undersøkelsene vi gjør i studien og derfor må kvinner i fruktbar alder bruke prevensjon godkjent av legen i studien. Hvis du tror du er gravid, informer derfor studieansvarlig så snart som mulig.

Oversikt over studiebesøkene

Visitt/besøk nr.	1 Uke -2	2 Uke 0 Randomisering	3 Uke 2	4 Uke 4	5 Uke 6	6 Uke 8	7 Uke 10	8 Uke 12	9 Uke 18	10 Uke 24
Informert samtykke	x									

Gjennomgang av kriterier for deltakelse	x									
Legeundersøkelse	x							x		x
Måling av vekt livvidde, blodtrykk og puls	x	x	x	x		x		x	x	x
Kostveiledning v/klinisk ernæringsfysiolog	x	x	x	x		x		x	x	
Kostholdsregistrering Inkludert complianse (hvordan du følger legens råd)	x						x			X
Rutine blodprøver fastende	x	x						x		X
Gjennomgang av symptomer eller ubehag	x	x	x	x		x		x	x	X
Gjennomgang og evt. Bytte av medisinbruk og kosttilskudd	x	x	x	x		x		x	x	X

Henting av fisk eller nøtter*		x	x	x	x	x	x	x	x	

*merknad: Mellom uke 12 og 18 og mellom uke 18 og 24 skal du komme til oss for henting av maten og samtale og oppdatering. Dette er pga langt mellomtid mellom de 3 siste besøkene. Dette er ikke merket i tabellen, men disse møtene vil være i studeuke 15 og 21.

Kapittel B – Ytterligere informasjon om personvern, biobank, økonomi og forsikring

Personvern

Det er en forutsetning for å delta, at du sier ja til insynsrett i gjeldende relevante journalopplysninger som er nødvendige for å kunne kontrollere at studieopplysningene stemmer overens med tilsvarende opplysninger i din journal. All informasjon vil bli behandlet konfidensielt. Fastlegen din blir vanligvis informert om din deltagelse hvis du ikke har noe i mot det. Personlige opplysninger om deg som kan være sensitive (for eksempel sykehistorie og medisinbruk), vil bli samlet inn og behandlet, men kun til forskningsformål i forbindelse med studien. Du vil ikke bli referert til ved navn eller bli identifisert i noen publikasjon, så dataene kan ikke spores tilbake til deg. Oslo universitetssykehus er databehandlingsansvarlig for studien.

Forskningsbiobank

Blod- og urinprøvene som blir tatt vil bli lagret i en forskningsbiobank. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet inngår i biobanken. Oslo Universitetssykehus ved prosjektleder Serena Tonstad er ansvarshavende for forskningsbiobanken. Biobanken planlegges å vare til 2025. Etter dette vil materiale bli ødelagt etter interne retningslinjer.

Innsynsrett og oppbevaring av materiale

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, vil det ikke samles inn flere opplysninger eller mer materiale. Opplysninger som allerede er registrert vil ikke bli slettet, dersom opplysningene allerede har inngått i analyse.

Finansiering

Denne studien er finansiert ved hjelp av midler fra Seksjon for preventiv kardiologi, Oslo Universitetssykehus og Extrastiftelsen via Nasjonalforeningen for Folkehelsen.

Forsikring

Vi forventer ikke at du skal få noen helseproblemer ved å delta i denne studien, men dersom din helse forverres som et resultat av deltagelse vil du kunne få erstatning. Du må ikke bevise at det var noen sin skyld. Dersom det viser seg at problemene oppstod som følge av studien, vil du få erstatning. Du er forsikret i henhold til Oslo universitetssykehus sine egne forsikringer.

Informasjon om resultatet av studien

Du har rett til å få informasjon om resultatet av studien. Legen i studien vil kunne fortelle deg dette når resultatene er klare.

Samtykke for deltagelse i studien

Jeg er villig til å delta i studien

(Signert og datert av deltager)

Deltagers navn med blokkbokstaver

Deltagers fødselsdato

Bekreftelse på at informasjon er gitt deltageren i studien

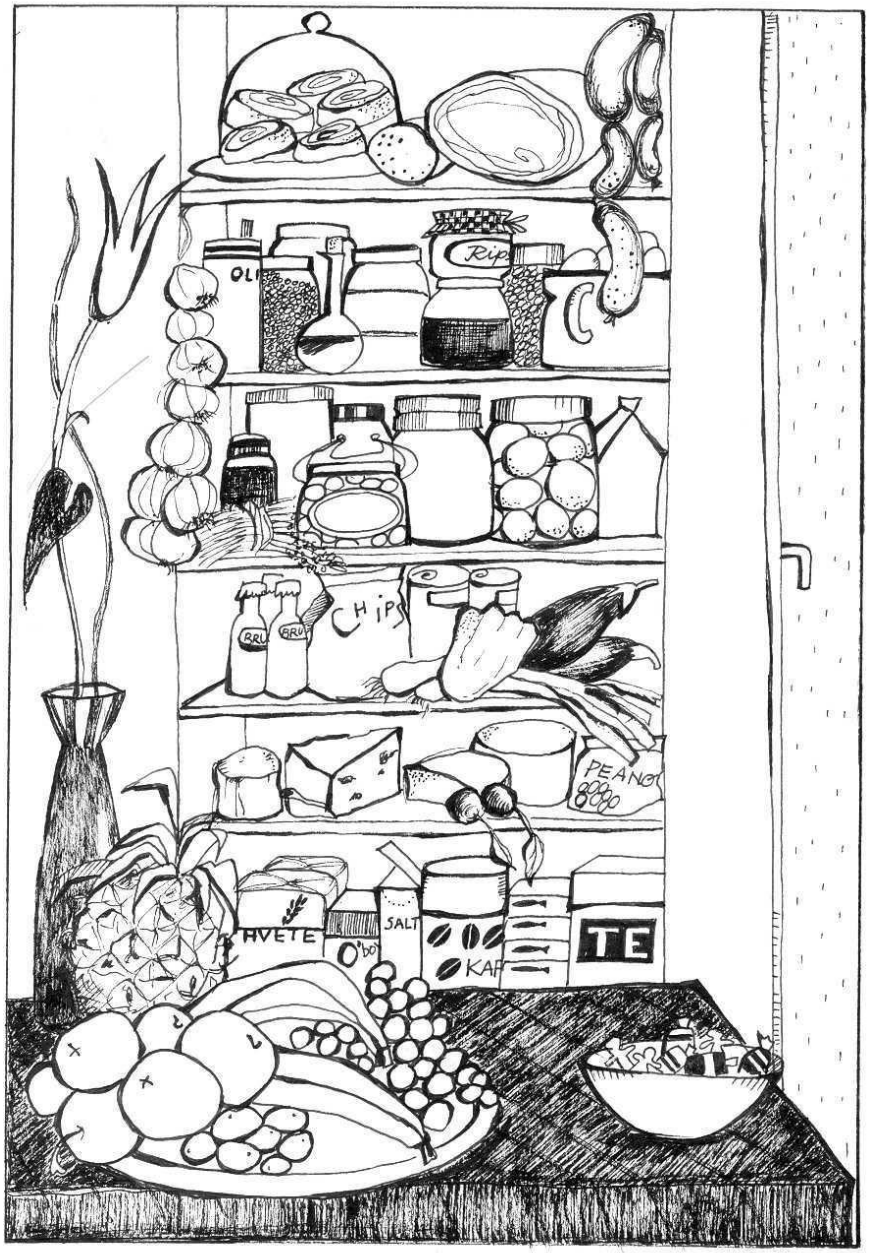
Jeg bekrefter å ha gitt skriftlig og muntlig informasjon om studien

---

(Signert og datert av lege)

NASJONALT FOLKEHELSEINSTITUTT

Kostholdet ditt



Veiledning for utfylling av kostskjemaet

I dette skjemaet spør vi hva du har spist det siste året. Dette innebærer at vi ber deg tenke tilbake på hva du har spist de 12 siste månedene.

Vi takker for at du vil hjelpe oss med denne undersøkelsen.

Skjemaet skal leses av en maskin. ***Det er derfor viktig at du legger vekt på følgende ved utfyllingen*****:**

- Bruk blå eller sort kulepenn
- I de små avkrysningsboksene setter du *et kryss* for det svaret som du mener passer best, slik: ☒.
- Du skal sette ett kryss på hver linje
- Skriver du feil, kan du kan ta bort krysset ved å fylle boksen helt, slik: ■ og deretter fylle i det riktige alternativet.

Eksempel:

Antall brødskiver med dette pålegg

Ost	per dag						eller	per uke			eller	per måned			
	6+	5	4	3	2	1		5-6	3-4	1-2		3	2	1	0
Brunost (Gudbrandsdalsost o.l.)	☐	☐	☐	☒	☐	■		☐	☐	☐		☐	☐	☐	☐

- Der hvor det står et + betyr dette "og flere enn". Eksempel: 6+ betyr 6 og flere enn 6
- Når du fyller ut skjemaet skal du tenke på hva du har spist det siste året og angi et gjennomsnitt.

Eksempel: Hvis du spiste torsk, sei mm til middag 1 gang i uken 6 måneder på rad, det første halve året, men ikke har spist torsk, sei med mer etter dette, har du totalt spist torsk og sei 24 ganger. I gjennomsnitt blir dette 2 gang per måned og du setter da kryss i boksen for 2 ganger per måned slik;

Antall middager

Middag med Torsk, sei, kolje, lyr	per uke 5+	4	3	2	1	eller	per måned 3	2	1	0
Makrell, sild	☐	☐	☐	☐	☐		☐	☒	☐	☐

- Enkelte steder kan du skrive tekst
  - Skriv tydelig
  - Skriv bare tekst når du blir bedt om det

Så snart du har fylt ut dette skjemaet, sender du det tilbake til oss i den vedlagte, frankerte svarkonvolutten.

Avdeling for miljømedisin
Nasjonalt folkehelseinstitutt
Postboks 4404 Nydalen
0403 Oslo

Oppgi dag, måned og år for utfylling av skjemaet: ☐☐ ☐☐ ☐☐☐☐ (skriv årstall med 4 tall, f.eks 2003)

dag måned år

Kostvaner

1. Hvordan vil du beskrive dine kostvaner det siste året?

Kostvaner	Sett bare ett kryss
1. I mitt kosthold inngår kjøtt og fisk	☐
2. Jeg unngår kjøtt, men spiser fisk	☐
3. Jeg unngår fisk, men spiser kjøtt	☐
4. Jeg er vegetarianer og inkluderer melkeprodukter og egg i kosten (ovolakto-vegetarianer)	☐
5. Jeg er vegetarianer og inkluderer melkeprodukter, men ikke egg i kosten (lakto-vegetarianer)	☐
6. Jeg er vegetarianer og utelater alle melkeprodukter og egg fra kosten (veganer)	☐

2.* Har du brukt økologiske matvarer det siste året? *(Sett bare ett kryss på hver linje.)

Økologisk matvare	Sjeldent/aldri	Noen ganger	Ofte	For det meste
1. Melk, melkeprodukter og ost	☐	☐	☐	☐
2. Brød og kornprodukter (f. eks. mel, müsli)	☐	☐	☐	☐
3. Egg	☐	☐	☐	☐
4. Grønnsaker	☐	☐	☐	☐
5. Frukt	☐	☐	☐	☐
6. Kjøtt	☐	☐	☐	☐

Antall måltider

3. Hvor ofte har du i gjennomsnitt spist følgende måltider per uke det siste året?
Et mellommåltid er et mindre måltid som for eksempel kan bestå av frukt, kjeks, bolle, yoghurt eller godteri. Mellommåltider som bare består av drikke skal ikke tas med da det blir spurt etter

drikke seinere. *(Sett bare ett kryss på hver linje.)*

	Antall måltider per uke							
	7	6	5	4	3	2	1	0
1. Frokost	☐	☐	☐	☐	☐	☐	☐	☐
2. Mellommåltid, formiddag	☐	☐	☐	☐	☐	☐	☐	☐
3. Formiddagsmat	☐	☐	☐	☐	☐	☐	☐	☐
4. Mellommåltid, ettermiddag	☐	☐	☐	☐	☐	☐	☐	☐
5. Middag	☐	☐	☐	☐	☐	☐	☐	☐
6. Mellommåltid, kveld	☐	☐	☐	☐	☐	☐	☐	☐
7. Kveldsmat	☐	☐	☐	☐	☐	☐	☐	☐
8. Nattmat	☐	☐	☐	☐	☐	☐	☐	☐

Brød, knekkebrød, kjeks

4. Hvor mange skiver brød/ knekkebrød/ kjeks har du spist i gjennomsnitt per dag/ uke det siste året? Når du svarer på spørsmålene, skal du tenke på brød til alle måltider i løpet av dagen. Et halvt rundstykke = 1 skive brød, 1 baguett = 4 skiver brød, 1 ciabatta = 3 skiver. *(Sett bare ett kryss på hver linje.)*

Brødtype	*Antall brødskiver* per dag										eller per uke			
	13+	9-12	8	7	6	5	4	3	2	1	5-6	3-4	1-2	0
1. Fint brød (loff, baguetter, ciabatta o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2. Mellomgrovt brød (kneipp, husholdn.brød)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
3. Grovt brød (fiber kneipp, rugbrød o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4. Klibrød, kli-knekkebrød, rugsprø	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
5. Knekkebrød, skonrokk grov o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

6. Smørbrødkjeks (Kaptein kjeks o.l.) ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ | ☐ ☐ ☐ ☐

5. Bruker du smør/margarin på brød/ knekkebrød/ kjeks?

☐ **Ja** ☐ **Nei** (gå til spørsmål 8)

6. Hvis du bruker smør/margarin, på hvor mange skiver i gjennomsnitt og hvilken type smør/margarin bruker du? *(Sett bare ett kryss på hver linje.)*

Type smør/margarin	Antall brødskiver per dag 13+	9-12	8	7	6	5	4	3	2	1	eller per uke 5-6	3-4	1-2	0
1. Smør/Bremyk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
2. Hard margarin (Per, Melange)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
3. Brelett	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
4. Myk margarin (Soft, Vita, Olivero o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
5. Lett margarin (Soft light, Vita lett, o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

7. Hvor tykt lag med smør/margarin smører du på brødskivene?

Rikelig ☐ **Middels** ☐ **Skrapet** ☐

Pålegg på brød, knekkebrød, kjeks

8. Hvor mange brødskiver med de følgende påleggstypene har du spist i gjennomsnitt det siste året? *(Sett bare ett kryss på hver linje.)*

Ost	Antall brødskiver med dette pålegg												
	per dag						eller per uke			eller per måned			
	6+	5	4	3	2	1	5-6	3-4	1-2	3	2	1	0
1. Brunost (Gudbrandsdalsost o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2. Brunost lettvarianter, prim	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
3. Hvit ost, kremost, smøreost	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4. Lettvarianter av hvit ost, smøreost	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
5. Muggoster (Camembert, Norzola o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
6. Annet ostepålegg	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fiskepålegg													
7. Kaviar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
8. Makrell/sardin i tomat	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
9. Sardin i olje	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
10.Røkt laks/ørret/makrell	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
11.Sild (sursild o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
12.Reker	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
13.Krabbe	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
14.Tunfisk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

15.Svolværpostei (postei av fiskelever/rogn)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
16.Annet fiskepålegg	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kjøttpålegg												
17.Magert kjøttpålegg (skinke, roast biff o..l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
18.Servelat, lammerull, kalverull	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
19.Salt pølse, spekepølse, salami	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
20.Kalkun,- og kyllingpålegg	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
21.Leverpostei	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
22.Annet kjøttpålegg	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	Antall brødskiver med dette pålegg											
Andre typer pålegg	per dag						eller per uke			eller per måned		
	6+	5	4	3	2	1	5-6	3-4	1-2	3	2	1	0
23.Salater med majones (rekesalat o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
24.Frokostsalat	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
25.Majones	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
26.Syltetøy	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
27.Honning	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
28.Peanøttsmør	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

29.Annet nøttepålegg (Nugatti o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
30.Annet søtt pålegg (Sjokade, Hapå o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
31.Vegetabilske posteier (Tartex o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
32.Frukt (banan, eple o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
33.Grønnsaker (Tomat, agurk o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Egg

9. Hvor mange egg har du spist i gjennomsnitt det siste året? Ta med egg til alle måltider!
Egg i bakeverk skal ikke tas med! ***(****Sett bare ett kryss på hver linje.)*

	per dag		eller per uke			eller per måned		
Egg	2+	1	5-6	3-4	1-2	2-3	1	0
Egg, stekt, kokt, eggerøre, omelett	☐	☐	☐	☐	☐	☐	☐	☐

Antall måsegg spist siste 12 måneder	0 ☐	1-5 ☐	6-10 ☐	mer enn 10 ☐

Frokostgryn, grøt

10. Hvor ofte har du spist frokostgryn eller grøt i gjennomsnitt det siste året?
Dersom du har spist disse matvarene til andre måltider enn frokost skal du også ta det med her.

(Sett bare ett kryss på hver linje.)

Grøt, frokostgryn	Hvor ofte per dag 2+	per dag 1	eller per uke 5-6	per uke 3-4	per uke 1-2	eller per måned 2-3	per måned 1	per måned 0
1. Usøtede kornblandinger (4-korn, All-Bran Flakes o.l.)	☐	☐	☐	☐	☐	☐	☐	☐
2. Søtet müsli og müsli med frukt, nøtter	☐	☐	☐	☐	☐	☐	☐	☐
3. Havregrøt, annen grøt	☐	☐	☐	☐	☐	☐	☐	☐
4. Cornflakes, Frosties o.l.	☐	☐	☐	☐	☐	☐	☐	☐
5. Sukker på frokostgryn/grøt	☐	☐	☐	☐	☐	☐	☐	☐
6. Syltetøy på frokostgryn/grøt	☐	☐	☐	☐	☐	☐	☐	☐

Drikke

11. Hvor mange glass/ kopper av følgende har du drukket i gjennomsnitt det siste året? Ta også med melk/ yoghurt til frokostgryn/ grøt. 1 krus = 1 glass = 2 kopper = 2,5 dl, ½ liters plastflaske = 2 glass *(Sett bare ett kryss på hver linje.)*

Melk og yoghurt		Hvor mange glass/beger per dag 8+	per dag 6-7	per dag 4-5	per dag 2-3	per dag 1	eller per uke 5-6	per uke 3-4	per uke 1-2	eller per måned 2-3	per måned 1	per måned 0
1. H-melk, kefir, kulturmelk	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2. Lettmelk	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
3. Ekstra lett lettmelk	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4. Skummet melk søt, sur	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Melk og yoghurt	Hvor mange glass/beger per dag 8+	per dag 6-7	per dag 4-5	per dag 2-3	per dag 1	eller per uke 5-6	per uke 3-4	per uke 1-2	eller per måned 2-3	per måned 1	per måned 0

5. Cultura, alle typer	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
6. Biola drikke, Biola yogh.	(1 glass/beger)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
7. Yoghurt, naturell/frukt	(1 glass/beger)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
8. Lettyoghurt	(1 glass/beger)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
9. Go'morgen yoghurt	(1 beger	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
10.Sjokolademelk, Litago	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
11.Soyamelk	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
12.Ris-, havremelk	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Hvor mange glass

Juice/ saft/ brus/ vann/ alkohol		per dag					eller per uke			eller per måned		
		8+	6-7	4-5	2-3	1	5-6	3-4	1-2	2-3	1	0
13. Appelsinjuice	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
14.Annen fruktjuice, most, nektar	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
15.Tomat- og grønnsaksjuice	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
16.Saft med sukker	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
17.Saft, kunstig søtet	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
18.Coca Cola/Pepsi med sukker	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
19.Annen brus med sukker	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
20.Coca Cola-light/Pepsi-light	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
21.Annen brus-light	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
22.Energidrikk, Battery o.l.	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
23.Springvann (vann fra kran)	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
24.Flaskevann, uten kullsyre	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
25.Farris, vann med kullsyre	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
26.Alkoholfritt øl, vørterøl, lettøl	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
27.Pilsnerøl	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

28.Vin	(1 glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
29.Brennevin, likør	(1 dram)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Hvor mange kopper/krus

		per dag					eller per uke			eller per måned		
Kaffe/ te		8+	6-7	4-5	2-3	1	5-6	3-4	1-2	2-3	1	0
30.Filterkaffe	(1 kopp)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
31.Pulverkaffe	(1 kopp)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
32.Kokekaffe/ presskanne kaffe	(1 kopp)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
33.Kaffe latte, cappucino	(1 kopp)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
34.Espresso	(1 kopp)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
35.Koffeinfri kaffe	(1 kopp)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
36.Fiken/ korn kaffe	(1 kopp)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
37.Te (vanlig te, Lipton fruktte o.l.)	(1 krus)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
38.Grønn te	(1 krus)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
39.Nypete, urtete	(1 krus)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

12. I hvor mange kopper kaffe og te bruker du melk/ fløte/ sukker.

	per dag					eller per uke			eller per måned		
Melk/ fløte/ sukker i kaffe og te	8+	6-7	4-5	2-3	1	5-6	3-4	1-2	2-3	1	0
1. Melk/ fløte i kaffe/ te	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2. Sukker/ honning i kaffe/ te	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
3. Kunstig søtstoff i kaffe/ te	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Middagsmat (varm mat)

Først ber vi deg svare på et generelt spørsmål om ulike grupper av varm mat. Deretter ber vi deg svare mer i detalj på de ulike typene varm mat du har spist det siste året.

Når du svarer på disse spørsmålene ber vi deg å tenke på både middagsmat og annen varm

mat du eventuelt spiser i løpet av dagen.

13. Hvor ofte har du i gjennomsnitt spist følgende type varm mat det siste året?
(Sett bare ett kryss på hver linje.)

Generelle spørsmål	per uke						eller	per måned			
	6+	5	4	3	2	1		3	2	1	0
1. Kjøtt og kjøttprodukter	☐	☐	☐	☐	☐	☐		☐	☐	☐	☐
2. derav grillet	☐	☐	☐	☐	☐	☐		☐	☐	☐	☐
3. Innmat	☐	☐	☐	☐	☐	☐		☐	☐	☐	☐
4. Kylling, kalkun	☐	☐	☐	☐	☐	☐		☐	☐	☐	☐
5. Fisk, fiskeretter, kokt, ovnsbakt	☐	☐	☐	☐	☐	☐		☐	☐	☐	☐
6. Fisk, fiskeretter, stekt	☐	☐	☐	☐	☐	☐		☐	☐	☐	☐
7. Vegetarretter	☐	☐	☐	☐	☐	☐		☐	☐	☐	☐

Mer detaljerte spørsmål om hver enkelt middagsrett.

14. Hvor ofte har du i gjennomsnitt spist følgende type varm mat det siste året?

Sett bare ett kryss på hver linje.

Hvor ofte

Middag med blandings-**produkter av kjøtt**	per uke						eller	per måned			
	6+	5	4	3	2	1		3	2	1	0
1. Kjøttpølser, medisterpølser	☐	☐	☐	☐	☐	☐		☐	☐	☐	☐
2. Grillpølser, wienerpølser	☐	☐	☐	☐	☐	☐		☐	☐	☐	☐

3. Kylling-, kalkunpølser	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4. Kjøtt-, medisterkaker, kjøttpudding	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
5. Hamburgere, karbonader	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
6. Kjøttdeig i saus el. gryteretter	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Middag med okse-/ kalvekjøtt										
7. Okse,- og kalvestek	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
8. Biff (indrefilet, mørbrad, entrecote)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
9. T-bone stek, kalvekotelett	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
10.Kjøttgryte, lapskaus, kjøttsuppe	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Middag med svinekjøtt										
11.Kotelett, nakkekotelett, skinkestek	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
12.Indrefilet, flatbiff	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
13.Sommerkotelett/hamburgerrygg	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
14.Flesk/ribbe, ”spare ribs”	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
15.Bacon	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
16.Gryterett	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Middag med lam/sau										

17.Lammestek/lammekotelett	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
18.Gryteretter med lam/sau (Fårikål o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Middag med viltkjøtt										
19.Reinsdyrstek	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
20.Stek av elg, hjort, rådyr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
21.Reinsdyrkaker/gryterett av reinsdyr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
22.Karbonader/gryterett (elg, hjort, rådyr)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Middag med innmat	Hvor ofte per uke						eller per måned			
	6+	5	4	3	2	1	3	2	1	0
23.Lever, nyre fra okse, gris	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
24.Lever, nyre fra sau	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
25.Lever, nyre fra vilt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
26.Blodmat, lungemos	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Middag med fjørfe										
27.Kylling- og kalkunfilet	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
28.Grillet kylling	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

29.Stekt/kokt kylling, høne og kalkun	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
30.Kyllingschnitzel, nuggets	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
31.Viltfugl (rype, orrfugl o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
32.Annet fjørfe (and, gås, struts)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Middag med fisk/ sjømat										
33.Torsk, sei, kolje, lyr (kokt/stekt/røkt)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
34.Makrell, sild (kokt/stekt/røkt)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
35.Laks, ørret	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
36.Flyndrefisker (kveite, rødspette o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
37.Tunfisk (f.eks. i salat)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
38.Abbor, gjedde, gjeddekaker	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
39.Annen fisk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
40.Fiskekaker, fiskepudding, fiskeboller	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
41.Fiskepinner, fiskepanetter, panert fisk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
42.Fiskegryte, -grateng, suppe med fisk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
43.Reker	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
44.Skjell	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

45.Krabbe	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
46.Rogn	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
47.Fiskelever	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
Pastaretter		
48.Pastarett med kjøtt (spaghetti med kjøttsaus, Lasagne o.l.)	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
49.Pastarett med fisk/reker/skjell	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
50.Pastarett med grønnsaker	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
51.Pastarett med bare tomatsaus/ketchup	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
52.Ost (Parmesan o.l.) på pastarett	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
Annen varm mat		
53.Pizza	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
54.Taco, burritos o.l.	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
55.Pannekaker	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
56.Grøt (ikke frokostgrøt)	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
57.Suppe, hjemmelaget og posesuppe	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐

Grønnsaksrett som hovedrett									
58.Bare med grønnsaker	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
59.Med bønner/linser	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
60.Med soyaprodukter (pølser, o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Tilbehør til varm mat

15. Hvor ofte har du i gjennomsnitt spist følgende matvarer det siste året?
(Sett bare ett kryss på hver linje.)

	Hvor ofte						
	per dag	eller	per uke		eller	per måned	
Poteter/ris/spaghetti	1	5-6	3-4	1-2	2-3	1	0
1. Poteter (kokte, bakte, potetstappe)	☐	☐	☐	☐	☐	☐	☐
2. Pommes frites, stekte poteter	☐	☐	☐	☐	☐	☐	☐
3. Potetstuing, gratinerte poteter	☐	☐	☐	☐	☐	☐	☐
4. Spaghetti, makaroni, nudler	☐	☐	☐	☐	☐	☐	☐
5. Ris	☐	☐	☐	☐	☐	☐	☐
6. Hirse, couscous o.l.	☐	☐	☐	☐	☐	☐	☐
Saus/tilbehør							
7. Smeltet meierismør	☐	☐	☐	☐	☐	☐	☐
8. Smeltet margarin	☐	☐	☐	☐	☐	☐	☐
9. Brun/hvit saus	☐	☐	☐	☐	☐	☐	☐
10.Bearnaisesaus o.l.	☐	☐	☐	☐	☐	☐	☐
11.Majones, remulade	☐	☐	☐	☐	☐	☐	☐
12.Seterrømme, Crème Fraîche	☐	☐	☐	☐	☐	☐	☐
13.Lettrømme	☐	☐	☐	☐	☐	☐	☐

14.Ketchup	☐		☐	☐	☐	☐	☐	☐
15.Sennep	☐		☐	☐	☐	☐	☐	☐

Type fett til matlaging

16. Hvor ofte har du brukt følgende typer fett i matlagingen det siste året?
(Sett bare ett kryss på hver linje.)

	Hvor ofte							
	per dag		eller	per uke		eller	per måned	
Type fett til matlaging	2+	1	5-6	3-4	1-2	2-3	1	0
1. Smør, meierismør	☐	☐	☐	☐	☐	☐	☐	☐
2. Bremyk, Smørgod	☐	☐	☐	☐	☐	☐	☐	☐
3. Melange, Per	☐	☐	☐	☐	☐	☐	☐	☐
4. Soft, soyamargarin (pakke, beger)	☐	☐	☐	☐	☐	☐	☐	☐
5. Olivero	☐	☐	☐	☐	☐	☐	☐	☐
6. Annen margarin	☐	☐	☐	☐	☐	☐	☐	☐
7. Soyaolje	☐	☐	☐	☐	☐	☐	☐	☐
8. Matolje, rapsolje	☐	☐	☐	☐	☐	☐	☐	☐
9. Olivenolje	☐	☐	☐	☐	☐	☐	☐	☐
10.Maisolje	☐	☐	☐	☐	☐	☐	☐	☐
11.Andre oljer	☐	☐	☐	☐	☐	☐	☐	☐

Grønnsaker

Først ber vi deg svare på et generelt spørsmål. Deretter ber vi deg svare mer i detalj om de enkelte grønnsakene du har spist.

17. Hvor ofte har du i gjennomsnitt spist grønnsaker det siste året? *(Sett bare ett kryss på hver linje.)*

Oversiktsspørsmål	Hvor ofte							
	per dag		eller per uke			eller per måned		
	2+	1	5-6	3-4	1-2	2-3	1	0
1. Rå grønnsaker (salat, råkost o.l.)	☐	☐	☐	☐	☐	☐	☐	☐
2. Grønnsaker i gryteretter,supper,wok o.l.	☐	☐	☐	☐	☐	☐	☐	☐
3. Kokte grønnsaker som tilbehør	☐	☐	☐	☐	☐	☐	☐	☐

Mer detaljert om hver enkelt grønnsak.

18. Hvor ofte har du spist følgende grønnsaker det siste året? (*Sett bare ett kryss på hver linje.)*

Grønnsaker	Hvor ofte							
	per dag		eller per uke			eller per måned		
	2+	1	5-6	3-4	1-2	2-3	1	0
1. Grønnsaksblandinger, frosne	☐	☐	☐	☐	☐	☐	☐	☐
2. Agurk	☐	☐	☐	☐	☐	☐	☐	☐
3. Aubergine	☐	☐	☐	☐	☐	☐	☐	☐
4. Avocado	☐	☐	☐	☐	☐	☐	☐	☐
5. Blomkål, rå	☐	☐	☐	☐	☐	☐	☐	☐

6. Blomkål, kokt/i gryteretter	☐	☐	☐	☐	☐	☐	☐	☐	
7. Brokkoli, rå	☐	☐	☐	☐	☐	☐	☐	☐	
8. Brokkoli, kokt/i gryteretter	☐	☐	☐	☐	☐	☐	☐	☐	
9. Bønner (grønne-, aspargesbønner)	☐	☐	☐	☐	☐	☐	☐	☐	
10.Erter	☐	☐	☐	☐	☐	☐	☐	☐	
11.Gulrot, rå	☐	☐	☐	☐	☐	☐	☐	☐	
12.Gulrot, kokt/i gryteretter	☐	☐	☐	☐	☐	☐	☐	☐	
13.Hodekål, rå	☐	☐	☐	☐	☐	☐	☐	☐	
14.Hodekål, kokt/stuing/i gryteretter	☐	☐	☐	☐	☐	☐	☐	☐	
15.Hvitløk	☐	☐	☐	☐	☐	☐	☐	☐	
16.Kålrot, rå	☐	☐	☐	☐	☐	☐	☐	☐	
17.Kålrot, kokt/stappe/i gryteretter	☐	☐	☐	☐	☐	☐	☐	☐	
18Løk/ purre/ vårløk, rå	☐	☐	☐	☐	☐	☐	☐	☐	
19.Løk/ purre/ vårløk, stekt/ i gryteretter	☐	☐	☐	☐	☐	☐	☐	☐	
20.Mais	☐	☐	☐	☐	☐	☐	☐	☐	
21.Paprika, rå	☐	☐	☐	☐	☐	☐	☐	☐	
22.Paprika i gryteretter	☐	☐	☐	☐	☐	☐	☐	☐	
23.Rosenkål, kokt/i gryteretter	☐	☐	☐	☐	☐	☐	☐	☐	
24.Salatblandinger, ferdig i pose	☐	☐	☐	☐	☐	☐	☐	☐	
25.Salat (bladsalater, issalat, kinakål o.l)	☐	☐	☐	☐	☐	☐	☐	☐	
26.Selleri , stilkselleri	☐	☐	☐	☐	☐	☐	☐	☐	
27.Sjampinjong, rå	☐	☐	☐	☐	☐	☐	☐	☐	
28.Sjampinjong, stekt/i gryteretter	☐	☐	☐	☐	☐	☐	☐	☐	
29.Skogsopp, annen sopp	☐	☐	☐	☐	☐	☐	☐	☐	
30.Spinat	☐	☐	☐	☐	☐	☐	☐	☐	
31.Squash (zucchini)	☐	☐	☐	☐	☐	☐	☐	☐	
32.Tomat	☐	☐	☐	☐	☐	☐	☐	☐	

33.Andre grønnsaker	☐	☐	☐	☐	☐	☐	☐	☐

19. Hvor ofte har du brukt dressing og annet tilbehør til salat og råkost det siste året?

(Sett bare ett kryss på hver linje.)

	Hvor ofte							
	per dag		eller per uke			eller per måned		
Dressing/ annet tilbehør	2+	1	5-6	3-4	1-2	2-3	1	0
1. Dressing (thousand island o.l.)	☐	☐	☐	☐	☐	☐	☐	☐
2. Lett dressing, yoghurt dressing	☐	☐	☐	☐	☐	☐	☐	☐
3. Oliven, sorte/ grønne	☐	☐	☐	☐	☐	☐	☐	☐
4. Fetaost	☐	☐	☐	☐	☐	☐	☐	☐
Hjemmelaget dressing								
5. med olje	☐	☐	☐	☐	☐	☐	☐	☐
6. uten olje	☐	☐	☐	☐	☐	☐	☐	☐
7. med rømme/yoghurt	☐	☐	☐	☐	☐	☐	☐	☐

20. Vektforhold av kjøtt/grønnsaker i gryteretter. *(Sett bare ett kryss på hver linje.)*

	Har ikke spist	Mer grønnsaker enn kjøtt	Like mye kjøtt og grønnsaker	Mer kjøtt enn grønnsaker
1. Gryteretter med helt kjøtt/fisk	☐	☐	☐	☐
2. Gryteretter med innmat	☐	☐	☐	☐
3. Gryteretter med kjøttdeig	☐	☐	☐	☐

Frukt

21. Hvor mange friske frukter har du spist i gjennomsnitt siden det siste året?

	per dag					eller per uke			eller per måned		
	8+	6-7	4-5	2-3	1	5-6	3-4	1-2	2-3	1	0
Frisk frukt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

22. Hvor ofte har du spist følgende friske frukter det siste året?

(Sett bare ett kryss på hver linje.)

		Hvor ofte									
		per dag				eller	per uke		eller	per måned	
Frisk frukt		4+	3	2	1	5-6	3-4	1-2	2-3	1	0
1. Appelsin, mandarin	(1 stykk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2. Banan	(1 stykk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
3. Druer	(8-10 stykk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4. Eple	(1 stykk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
5. Fersken, nektarin	(1 stykk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
6. Grapefrukt	(½ stykk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
7. Jordbær	(¼ kurv)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
8. Andre bær (blåbær o.l)	(¼ kurv)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
9. Mango	(½ stykk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
10.Melon	(1 skive)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
11.Papaya	(½ stykk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
12.Plomme	(1 stykk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
13.Pære	(1 stykk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
14.Annen frukt		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

***23.* Hvor ofte har du i gjennomsnitt spist tørket frukt og nøtter det siste året?**
(*Sett bare ett kryss på hver linje.)*

	Hvor ofte									
	per dag				eller	per uke		eller	per måned	
Tørket frukt /nøtter	4+	3	2	1	5-6	3-4	1-2	2-3	1	0
1. Aprikoser	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2. Rosiner	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

3. Svisker, fiken, dadler	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4. Peanøtter	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
5. Mandler, hasselnøtter,cashewnøtter o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Dessert, is, kaker, godteri

24. . Hvor ofte har du i gjennomsnitt spist dessert og is siden det siste året?

(Sett bare ett kryss på hver linje.)

	Hvor ofte								
	per dag		eller	per uke			eller	per måned	
Dessert/is	2+	1		5-6	3-4	1-2		2-3	1	0
1. Puddinger (sjokolade, karamell o.l.)	☐	☐		☐	☐	☐		☐	☐	☐
2. Hermetisk frukt, fruktgrøt	☐	☐		☐	☐	☐		☐	☐	☐
3. Fruktsalat med frisk frukt	☐	☐		☐	☐	☐		☐	☐	☐
4. Fløteis	☐	☐		☐	☐	☐		☐	☐	☐
5. Yoghurtis, lettis	☐	☐		☐	☐	☐		☐	☐	☐
6. Saftis, sorbet	☐	☐		☐	☐	☐		☐	☐	☐
7. Vaniljesaus	☐	☐		☐	☐	☐		☐	☐	☐
8. Pisket krem, fløte	☐	☐		☐	☐	☐		☐	☐	☐

25. **Hvor ofte har du i gjennomsnitt spist kaker, boller og vafler det siste året?**
(Sett bare ett kryss på hver linje.)

		Hvor ofte									
		per dag				eller	per uke			eller	per måned	
Kaker, boller		4+	3	2	1		5-6	3-4	1-2		2-3	1	0
1. Boller, julekake o.l.	(1 stykke)	☐	☐	☐	☐		☐	☐	☐		☐	☐	☐
2. Wienerbrød -stang o.l.	(1 stykke)	☐	☐	☐	☐		☐	☐	☐		☐	☐	☐
3. Smultring, fyrstekake,													

formkake	(1 stykke)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4. Vafler	(1 plate)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
5. Sjokoladekake, bløtkake	(1 stykke)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
6. Søt kjeks, kakekjeks	(1 stykke)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

26. Hvor ofte har du i gjennomsnitt spist godteri det siste året?
(Sett bare ett kryss på hver linje.)

	Hvor ofte									
	per dag				eller	per uke		eller	per måned	
Godteri og snacks	4+	3	2	1	5-6	3-4	1-2	2-3	1	0
1. Ren sjokolade	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2. Sjokolade med nøtter o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
3. Karameller, konfekt, lakris	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4. Smågodt, seigmenn	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
5. Pastiller med sukker	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
6. Patiller uten sukker	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
7. Marsipan	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
8. Potetgull/skruer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
9. Popcorn	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
10.Saltstenger, lettsnacks o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Andre matvarer

27. Fordi det er vanskelig å få spurt om alle matvarer som du kan ha spist det siste året ber vi deg nedenfor skrive navnet *på matvarer som du kan ha spist og som det ikke har blitt spurt om.*

Hvor ofte

Andre matvarer		per dag						eller per uke			eller per måned	
		6+	5	4	3	2	1	5-6	3-4	1-2	2-3	1
Navn:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Navn:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Navn:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Navn:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Navn:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Genmodifisert mat

28. I mange land, bl.a. USA, England, og Frankrike, tillater man salg av genmodifiserte matvarer. De fleste europeiske land krever merking ved salg av genmodifiserte matvarer. Vi ønsker å vite om du har spist genmodifiserte matvarer eller matvarer med genmodifiserte ingredienser på reiser eller i Norge det siste året?

☐ **Ja** ☐ **Nei** ☐ **Vet ikke**

29. Hvis ja, ber vi deg skrive navnet på de genmodifiserte matvarene du kjenner til at du har spist

Genmodifiserte matvarer		Hvor ofte per dag 6+	5	4	3	2	1	eller per uke 5-6	3-4	1-2	eller per måned 2-3	1
Navn:		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Navn:		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Navn:		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Navn:		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Navn:		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Varm mat fra kiosk, bensinstasjon eller gatekjøkken

30. Hvor ofte har du i gjennomsnitt spist varm mat fra kiosk, bensinstasjon eller gatekjøkken det siste året?

Mat fra	per dag 4+	2-3	1	eller per uke 5-6	3-4	1-2	eller per måned 2-3	1	0
1. Kiosk	☐	☐	☐	☐	☐	☐	☐	☐	☐
2. Bensinstasjon	☐	☐	☐	☐	☐	☐	☐	☐	☐
3. Gatekjøkken, McDonald's o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐

Kostendringer i livet ditt

31. Nedenfor ber vi deg angi om du spiser/drikker mer, mindre eller samme mengde sammenliknet med for 30 år siden, eventuelt da du var barn (før du fylte 18 år)

Matvare	Spiste/drakk det heller ikke før	Som før	Mer	Mindre	Sluttet helt

1. Krabbe	☐	☐	☐	☐	☐
2. Reker	☐	☐	☐	☐	☐
3. Skjell (for eksempel blåskjell)	☐	☐	☐	☐	☐
4. Fiskelever (ikke tran)	☐	☐	☐	☐	☐

Matvare	Spiste/drakk det heller ikke før	Som før	Mer	Mindre	Sluttet helt
5. Tunfisk eller kveite	☐	☐	☐	☐	☐
6. Flyndre/annen flatfisk	☐	☐	☐	☐	☐
7. Gjedde(kaker) eller abbor	☐	☐	☐	☐	☐
8. Annen ferksvannsfisk	☐	☐	☐	☐	☐
9. Reinsdyr	☐	☐	☐	☐	☐
10.Sau/lammekjøtt	☐	☐	☐	☐	☐
11. Lever eller nyre fra vilt	☐	☐	☐	☐	☐
12.Viltvoksende sopp	☐	☐	☐	☐	☐
13. Måkeegg	☐	☐	☐	☐	☐

Kosttilskudd

39. Bruker du eller har du brukt kosttilskudd det siste året? ☐ **Ja** ☐ **Nei**

40. Hvis ja, ber vi deg å angi hvilken type og mengde nedenfor

(ts = teskje, bs = barneskje, ss = spiseskje)

Flytende kosttilskudd	Antall ganger per uke 7 6 5 4 3 2 1 <1 0	Mengde per gang 1 ts 1bs 1ss
1. Tran	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐
2. Omega-3 tran	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐
3. Sanasol	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐
4. Biovit	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐
5. Flytende jernmixtur (Floradix o.l.)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐
Annet flytende kosttilskudd		
Navn: Produsent:	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐
Navn: Produsent:	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐
Kapsler/tabletter	Antall ganger per uke 7 6 5 4 3 2 1 <1 0	Antall per gang 1 2 3 4+
6. Trankapsler	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
7. Fiskeoljekapsler	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐
8. Vitaplex	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐

9. Kostpluss/Nyco plus multi	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
10.Nyco plus folsyre 0,4 mg	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
11.Spektro (Solaray)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
12.Hemofer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
13.Duroferon duretter, Ferro retard	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Andre kosttilskudd **Skriv navn** **Produsent**													
Navn: Produsent:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Navn: Produsent:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Navn: Produsent:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Navn: Produsent:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

Vi vet at du har brukt tid på å fylle ut dette skjemaet og vi takker for hjelpen. For at vi skal få en vurdering av tidsbruken ved utfylling av skjemaet, ville det være fint om du kunne angi tiden du har brukt.

Jeg har brukt omtrent ☐☐☐ minutter til å fylle ut skjemaet.

Tusen takk for hjelpen!

14. Papers I-IV

METABOLIC SYNDROME AND RELATED DISORDERS
Volume 14, Number 8, 2016

Pp. 410–415
DOI: 10.1089/met.2016.0031

Associations of Circulating PYY_{3-36} Concentrations with Metabolic Syndrome in Extremely Obese Subjects

Sasa Dusanov, MD,[1] Kari Brandal, MSc,[2] Eli Heggen, MD,[1] and Serena Tonstad, MD, PhD[1]

Abstract

Background: The gut hormone peptide YY_{3-36} (PYY_{3-36}) plays major roles in regulation of appetite and energy metabolism, mediates beneficial effects of bariatric surgery, and may be a potential weight-reducing and glucose-modulating therapy. Obesity may influence the metabolic expression of circulating PYY_{3-36} and metabolic markers. We studied the relationship of PYY_{3-36} concentrations with metabolic syndrome (MetSyn) components, lipids, insulin resistance, and inflammatory biomarkers in subjects with extreme obesity.
Methods: We measured MetSyn components and PYY_{3-36}, lipids, hormones, homeostasis model assessment (HOMA) index, and inflammatory biomarkers in consecutively referred patients (180 women and 111 men) aged 18–78 years with body mass index (BMI) $\geq 40\,kg/m^2$. Associations of PYY_{3-36} to components, insulin resistance, and biomarkers were examined with partial correlations and linear regression.
Results: PYY_{3-36} concentrations were not related to MetSyn components, HOMA index, or to inflammatory biomarker or leptin concentrations. PYY_{3-36} concentrations correlated with systolic blood pressure ($r = 0.21$; $P < 0.0001$) after adjustment for age and gender. In linear regression analysis, PYY_{3-36} concentrations were associated with systolic blood pressure after adjustment for age, gender, and central obesity in the entire sample (Beta 0.21; 95% CI 0.09–0.34) as well as in subjects not taking blood pressure-lowering medication (Beta 0.19; 95% CI 0.04–0.36). These associations were not statistically significant in the small subset of participants (22%) with type 2 diabetes.
Conclusions: In extremely obese patients, fasting PYY_{3-36} concentrations were linked to systolic blood pressure, but not to other components of MetSyn, suggesting divergence between pathways of blood pressure and glucose/body weight regulation. However, this finding will need to be further investigated.

Keywords: metabolic syndrome, obesity, PYY_{3-36}, diabetes

Background

Metabolic syndrome (MetSyn), a constellation of abdominal obesity, hypertension, dyslipidemia, and dysglycemia, contributes to cardiovascular diseases (CVD) and type 2 diabetes directly and by creating a proinflammatory milieu.[1] Paralleling the obesity epidemic, MetSyn affects one-third of the United States and other populations,[2] yet in developed countries, CVD incidence continues to decrease, due, in part, to improvements in other risk factors.[3] Persons with very high body mass index (BMI $\geq 40\,kg/m^2$), a condition here referred to as extreme obesity, bear a large burden of morbidity and mortality. Extreme obesity has increased relatively more markedly in the population than lesser forms of obesity.[4] The musculoskeletal, health, and psychosocial consequences of extreme obesity are evident. However, cardiometabolic aberrations do not always correlate with the degree of obesity with some extremely obese individuals exhibiting no signs of lipid disturbance or insulin resistance.[5] In one study, MetSyn patients with extreme obesity had a lower waist–hip ratio and free fatty acid and triglyceride concentrations than patients with MetSyn who were not morbidly obese.[6] On the other hand, another study found that fat distribution predicted lipid abnormalities in women even with BMI $\geq 40\,kg/m^2$ and dysregulated glucose is very common in this group.[7,8]

Gastrointestinal hormones play important roles in the control of satiation, appetite, and energy expenditure and may exhibit impaired effects in obesity.[9] Among these hormones, peptide tyrosine-tyrosine (PYY_{1-36}) is released by endocrine L-cells in the small bowel and colon and cleaved by the enzyme dipeptidyl peptidase-4 to the major circulating form that is more bioactive, PYY_{3-36}.[10] Following a meal, circulating

[1]Section for Preventive Cardiology, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway.
[2]Rimfrost AS, Fosnavåg, Norway.

concentrations of PYY_{3-36} start to rise within ~15 min and remain elevated for several hours.[11] PYY_{3-36} inhibits food intake directly due to its high affinity for the presynaptic inhibitory Y2R neurons in the hypothalamic arcuate center and Y2R in afferent vagal fibers.[10,11] Baseline concentrations are influenced by adiposity, age, gender, and lifestyle, while postprandial concentrations are stimulated by caloric load and macronutrient composition, as well as acute exercise.[10–12] PYY_{3-36} responses are blunted in obesity and enhanced following bariatric surgery, in part, explaining the weight and, possibly, glucose reducing effects of surgery.[10]

Bariatric surgery remains currently the only effective treatment option for patients with extreme obesity, yet not all qualify or wish for surgery. New treatments are needed to bridge the gap between lifestyle changes, which may not be effective and no treatment, which puts patients at high risk of CVD and type 2 diabetes. Gut hormones represent potential therapeutic agents against obesity, as exemplified by the approval of high-dose liraglutide as an antiobesity agent. Studies demonstrated that administration of PYY_{3-36} led to a dose-dependent reduction in food intake indicating that PYY_{3-36} would be a potential antiobesity therapy.[13,14] However, we are aware of one cross-sectional study that found adverse associations between high PYY_{3-36} concentrations and metabolic risk factors in patients with coronary artery disease.[15]

Understanding the pathophysiology of PYY_{3-36} in patients with extreme obesity, its relationship to MetSyn, and influences of PYY_{3-36} on risk factors for CVD and type 2 diabetes is imperative. In this study, we studied cross-sectional associations of PYY_{3-36} and components of MetSyn, lipids, insulin resistance, and inflammatory biomarkers in patients with BMI ≥40 kg/m^2, herein defined as extreme obesity.

Materials and Methods

A total of 291 consecutive patients aged 18–78 years with BMI ≥40 kg/m^2 (180 women and 111 men) referred to the Preventive Cardiology Clinic at Oslo University Hospital, Oslo, Norway, participated in the study between April 2005 and December 2010. The participation rate was over 95%. The study conformed to the Helsinki Declaration and was evaluated by the Regional Ethics Committee.

After written informed consent, participants completed a health questionnaire and underwent anthropometric measurements. A constant tension body tape measure was used to determine waist and hip circumferences. Waist circumference was measured at midpoint between the inferior costal margin and the highest point of the iliac crest, and hip circumference was measured at the widest point around the hips. Height was measured using a stadiometer and recorded to the nearest centimeter. Patients were weighed to the nearest 1.0 kg using a calibrated mobile electronic scale (Seca 720; Medical Scales and Measuring Systems). BMI was calculated in accordance with the Quetelet's formula: Body weight in kilograms divided by the square of body height in meters (kg/m^2).

The participants' blood pressure was measured with an automatic blood pressure monitor (52000 Series Vital Signs Monitor; Welch Ally). Measurements were carried out with participants seated and having rested for 5 min prior using an appropriate cuff size. The average of the two lowest measurements was recorded.

All subjects were stratified by number of MetSyn components (from 1 to 5) as follows: elevated waist circumference ≥102 cm for men and ≥88 cm for women, systolic blood pressure ≥130 mmHg and/or diastolic ≥85 mmHg, or/and use of medication for hypertension, triglycerides ≥1.7 mM, high-density lipoprotein cholesterol (HDL-C) ≤1.0 mM for men or ≤ 1.3 mM for women, and fasting glucose ≥5.6 mM. Subjects with fasting glucose ≥7.0 mM or HbA1c ≥6.5%, who had known type 2 diabetes, or used antidiabetic drugs were grouped together as the type 2 diabetes subgroup (22% of the sample). Of the entire sample, use of blood pressure-lowering medication was reported among 31.3%; among those without diabetes, 26.1% used medication versus 49.2% of those with diabetes. Statin use was recorded in 8.9% of the entire sample; among those without diabetes in 5.3% and of those with diabetes in 21.5%.

Laboratory analyses

Participants fasted overnight for at least 10 hr, before providing blood samples between 8:00 and 11:00 a.m. the following day. Following immediate centrifugation, serum samples were stored at −80°C. Analyses were performed at Oslo University Hospital (Clinical Chemistry Laboratory at Ullevål and Endocrine Laboratory at Aker). Total cholesterol, HDL-C, triglyceride, glucose, and CRP concentrations were measured on the automated analyzer Cobas Integra 800 (Roche Diagnostics). LDL-cholesterol was calculated using Friedewald's formula. Apolipoprotein B was determined with an immunoturbidimetric assay on an automated analyzer (Cobas Tinaquant 917, Roche/Hitachi; Roche Diagnostics). White blood cells were analyzed using Sysmex XE 2100 (Sysmex). Serum ferritin was determined by an ADIVA Centaur analysis (ADIVA Centaur; Siemens Healthcare Diagnostics, Inc.).

PYY_{3-36} and insulin assays were carried out between November 2010 and May 2011 on frozen samples. Serum PYY_{3-36} was measured using a radioimmunoassay kit, which utilizes an antibody that only recognizes the $_{3–36}$ form of human PYY. The intra-assay and interassay variations of coefficients were less than 15%, and the recovery was 85%–129% by the linear range of the assay. The detection limit of the assay was 14 pg/mL (100 µg sample size). The assay had a specificity of 100% for human serum $PYY_{3-36.}$ Insulin was determined by noncompetitive immunofluorometric assay, using an AutoDelfia 1235 Automatic Immunoassay System (H1855-21291) (Perkin Elmer, Inc.). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated to estimate insulin resistance.[16]

Serum leptin was measured using a human serum leptin radioimmunoassay (Luminex). The intra-assay and interassay variations of coefficients were <10%, and the recovery was 103%–105% by the linear range of assay. The detection limit of the assay was 0.5 ng/L (100 µL sample size). The assay had a specificity of 100% for human serum leptin.

Statistical analyses

Statistical analyses were performed using SPSS 21 (SPSS, Inc.). Skewed variables, including PYY_{3-36}, triglycerides, glucose, C-reactive protein, ferritin, white blood cells, alanine transaminase, insulin, leptin, and homeostasis model assessment (HOMA) index, were log-transformed before analysis.

Table 1. BMI, Metabolic Syndrome Components, Lipids, Inflammatory Biomarkers, $PYY_{3–36}$, Hormones, and Homeostatic Assessment Index of Insulin Resistance Stratified by Number of Metabolic Syndrome Components and Diabetes ($N=291$)[a]

	Number of components					*Presence of type 2 diabetes*		
Parameter	*One or two* (n = *75*)	*Three* (n = *75*)	*Four* (n = *57*)	*Five* (n = *19*)	P	*No diabetes* (n = *226*)	*Diabetes* (n = *65*)	P
Age, years	37.4 (12.2)	40.1 (11.3)	40.6 (10.4)	46.7 (8.7)	<0.0001	39.9 (11.4)	41.1 (9.3)	0.007
Female, *n* (%)	52 (69.3)	45 (60.0)	37 (64.9)	6 (31.6)	0.010	140 (61.9)	40 (61.5)	
BMI, kg/m^2	45.1 (4.2)	45.5 (4.0)	45.4 (4.7)	44.9 (4.8)	1.0	45.3 (4.3)	46.6 (5.1)	0.035
Metabolic syndrome components								
Waist circumference, cm	126.5 (14.6)	131.2 (13.1)	130.6 (13.8)	134.7 (13.8)	0.003	129.8 (13.9)	135.1 (15.6)	0.009
Systolic BP, mmHg	126.8 (15.5)	139.3 (20.0)	137.0 (14.3)	138.2 (9.1)	<0.0001	134.4 (17.2)	136.6 (13.8)	0.4
Diastolic BP, mmHg	82.5 (10.3)	88.0 (11.6)	87.8 (10.4)	87.5 (6.6)	<0.0001	86.1 (10.8)	87.7 (8.8)	0.3
HDL-C, mM	1.4 (0.3)	1.3 (0.3)	1.1 (0.2)	0.9 (0.1)	<0.0001	1.2 (0.3)	1.1 (0.3)	<0.0001
Triglycerides, mM	1.2 (0.4)	1.6 (0.8)	2.3 (0.9)	3.2 (1.5)	<0.0001	1.8 (1.0)	2.1 (1.2)	0.004
Glucose, mM	5.0 (0.4)	5.6 (0.7)	5.6 (0.6)	6.0 (0.4)	<0.0001	5.4 (0.6)	8.7 (3.2)	<0.0001
Other lipids								
LDL-C, mM	3.2 (0.9)	3.3 (1.0)	3.5 (0.9)	3.2 (1.5)	0.4	3.3 (0.9)	3.0 (1.1)	0.043
Apolipoprotein B, g/L	0.9 (0.2)	1.0 (0.2)	1.1 (0.2)	1.1 (0.2)	<0.0001	1.0 (0.2)	1.0 (0.3)	1.0
Inflammatory biomarkers								
C-reactive protein, mg/L	12.4 (21.2)	10.4 (9.9)	10.9 (17.7)	5.9 (3.0)	0.6	10.8 (16.2)	10.4 (8.8)	0.3
Ferritin, µg/L	117.4 (136.7)	135.5 (125.4)	147.6 (135.8)	188.7 (129.1)	0.2	137.1 (132.7)	121.1 (104.2)	0.6
White blood cells, $\times 10^9$/L	7.1 (1.9)	8.0 (6.5)	7.4 (1.8)	7.1 (2.0)	0.7	7.5 (4.1)	7.6 (1.8)	0.2
Hormones								
Fasting $PYY_{3–36}$, pg/mL	95.8 (65.6)	120.5 (136.4)	129.0 (147.8)	120.2 (107.4)	0.4	114.4 (118.7)	106.8 (71.7)	0.9
Insulin, pM	90.5 (67.9)	126.4 (67.0)	127.8 (55.4)	163.9 (64.7)	<0.0001	117.1 (67.5)	134.7 (86.6)	0.2
Leptin, ng/mL	60.7 (29.0)	63.4 (37.7)	53.7 (31.8)	51.6 (27.6)	0.1	59.1 (32.8)	52.3 (34.7)	0.052
HOMA-IR index	2.9 (2.3)	4.5 (2.6)	4.6 (2.2)	6.2 (2.3)	<0.0001	4.1 (2.5)	7.2 (4.5)	<0.0001

[a]Mean (SD) shown. For insulin 4 missing, for LDL-C 11 missing, for apolipoprotein B and ferritin 1 missing, for white blood cells 3 missing.
BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; SD, standard deviation.

Categorical and continuous data are presented as counts and percentages, or mean ± standard deviation (SD), respectively. One-way analysis of variance (ANOVA) or independent *t*-tests were performed to compare components of MetSyn and other risk factors across groups, as appropriate. Partial correlation coefficients (corrected for age and gender) were used to analyze relationships between $PYY_{3\text{-}36}$ and risk factors. Linear regression analysis was used to establish independent relationships between $PYY_{3\text{-}36}$ concentrations and risk factors that showed statistically significant partial correlations. Adjustment was made for age, gender, and waist circumference. Two-sided *P*-values of <0.05 were considered statistically significant.

Results

Subject characteristics are presented in Table 1. Of the total of 291 subjects (180 women and 111 men), 23% were cigarette smokers. Smoking was not related to $PYY_{3\text{-}36}$ concentrations; additionally, $PYY_{3\text{-}36}$ concentrations did not differ according to use of statins (data not shown). Weight loss was attempted one or more times among 61% of those with one to two MetSyn components, 59% of those with three components, 47% of those with four components, 63% of those with five components, and 55% of those with type 2 diabetes ($P = 0.4$).

The range of BMI was between 40 and 74 kg/m^2. In subjects without type 2 diabetes, BMI did not differ according to the number of MetSyn components, although age and the proportion of males increased with increasing number of components. Each of the MetSyn component levels worsened with increasing number of MetSyn components, as did apolipoprotein B, insulin concentrations, and HOMA-IR index. Participants with type 2 diabetes were slightly older than their counterparts without type 2 diabetes, and had higher BMI, waist circumference, triglyceride and glucose concentrations and HOMA-IR, and lower HDL-C and low-density lipoprotein cholesterol (LDL-C) concentrations. $PYY_{3\text{-}36}$ concentrations did not differ according to MetSyn components or the presence of type 2 diabetes.

Correlational analyses shown in Table 2 found statistically significant relationships between $PYY_{3\text{-}36}$ concentrations and systolic blood pressure in the entire sample and in those not taking blood pressure-lowering medication. This finding was not statistically significant in the subgroup with type 2 diabetes. No other statistically significant correlations were observed between $PYY_{3\text{-}36}$ concentrations and components of MetSyn, lipids, and leptin, or insulin concentrations or HOMA-IR index.

In multiple linear regression analysis, we found significant associations between concentrations of $PYY_{3\text{-}36}$ and systolic blood pressure in the entire sample, and in the subgroup without type 2 diabetes after adjustment for age, gender, and central obesity (Table 3). The associations between $PYY_{3\text{-}36}$ and systolic blood pressure were also statistically significant in the subgroup of patients not using antihypertensive medication.

Discussion

Our main findings were that fasting, unstimulated $PYY_{3\text{-}36}$ concentrations did not relate to number of MetSyn components or to single components of MetSyn with the following exceptions:

TABLE 2. PARTIAL CORRELATIONS BETWEEN $PYY_{3\text{–}36}$ CONCENTRATIONS AND BMI, NUMBER OF METABOLIC SYNDROME COMPONENTS, COMPONENTS OF METABOLIC SYNDROME, LIPIDS, INFLAMMATORY BIOMARKERS, HORMONES, AND HOMA-IR INDEX, ADJUSTED FOR AGE AND GENDER[a]

	All (N = 291)		*No type 2 diabetes (n = 226)*		*With type 2 diabetes (n = 65)*	
	Coefficient	P	*Coefficient*	P	*Coefficient*	P
BMI	0.070	0.2	0.098	0.1	0.007	1.0
# Of MetSyn components	0.031	0.6	0.051	0.4	-	
Components of MetSyn						
Waist circumference	0.093	0.1	0.107	0.1	0.059	0.6
Systolic BP (all)	0.205	<0.0001	0.200	0.003	0.205	0.1
Diastolic BP (all)	0.062	0.3	0.039	0.6	0.142	0.3
Systolic BP (no medication)	0.178	0.012	0.182	0.020	0.153	0.4
Diastolic BP (no medication)	0.056	0.4	0.051	0.5	0.135	0.5
HDL-C	–0.014	0.8	0.003	1.0	–0.069	0.6
Triglycerides	0.052	0.4	0.051	0.4	0.047	0.7
Fasting glucose	0.088	0.1	0.082	0.2	0.226	0.1
Other Lipids						
LDL-C	–0.010	0.9	–0.007	0.9	–0.031	0.8
Apolipoprotein B	–0.013	0.8	–0.009	0.9	–0.032	0.8
Inflammatory biomarkers						
C-reactive protein	0.042	0.5	0.060	0.9	–0.029	0.8
Ferritin	0.036	0.5	0.022	0.7	0.076	0.6
White blood cells	–0.011	0.8	0.001	1.0	–0.082	0.5
Hormones						
Insulin	0.039	0.5	0.098	0.1	–0.165	0.2
Leptin	–0.014	0.8	0.010	0.9	–0.085	0.5
HOMA-IR index	0.068	0.3	0.105	0.1	–0.055	0.7

[a]For insulin 4 missing, for LDL-C 11 missing, for apolipoprotein B and ferritin 1 missing, for white blood cells 3 missing.
MetSyn, metabolic syndrome.

Table 3. Adjusted Associations of $PYY_{3–36}$ to Systolic Blood Pressure in Participants With and Without Type 2 Diabetes According to Use of Blood Pressure-Lowering Medication[a]

	Systolic BP	*Systolic BP*	*Systolic BP*
All	All ($N=291$)	No type 2 diabetes ($n=226$)	With type 2 diabetes ($n=65$)
B (unstandardized coefficient)	0.004	0.004	0.005
95% CI for B	0.002–0.007	0.001–0.007	–0.001–0.010
Beta (standardized coefficient)	0.211	0.207	0.205
95% CI for Beta	0.087–0.335	0.059–0.343	–0.054–0.524
P	0.001	0.006	0.1
No BP-lowering medication	$n=200$	$n=167$	$n=33$
B	0.004	0.004	0.005
95% CI for B	0.001–0.007	0.001–0.007	–0.007–0.017
Beta	0.192	0.193	0.161
95% CI for Beta	0.036–0.356	0.018–0.360	–0.364–0.862
P	0.017	0.030	0.4

[a]Adjusted for age, gender, and waist circumference.
BP, blood pressure.

PYY_{3-36} concentrations were linked to systolic blood pressure in the entire sample and in participants not taking blood pressure-lowering medication. Furthermore, PYY_{3-36} concentrations were not related to inflammatory biomarkers or lipids (LDL-C, apolipoprotein B), leptin concentrations, or to HOMA-IR index in extremely obese subjects.

Genetic data indicate that common variation at the PYY locus influences not only PYY concentrations but also multiple hereditable MetSyn traits, including BMI and lipids.[17] However, this study did not account for the influence of PYY variation on PYY_{3-36} or PYY_{1-36} concentrations and their potentially divergent associations with Metsyn.[10,17] The two native forms of PYY, PYY_{1-36} and its metabolite PYY_{3-36}, differ in their actions on appetite and glucose homeostasis, due to different binding affinities on the YR receptors.[10] PYY_{3-36}, but not PYY_{1-36}, improves glucose tolerance, although these effects are difficult to distinguish from the effects of PYY_{3-36} on feeding.[10,18] Recently, the importance of measuring the biologically relevant form of PYY, namely PYY_{3-36}, as done in this study, was emphasized.[10]

In individuals with extreme obesity, our finding of no relationship between PYY_{3-36} concentrations and MetSyn components, insulin resistance, or presence of type 2 diabetes is consistent with some studies and differs from others. A study of patients with coronary artery disease, who were not selected for obesity, found that high fasting PYY_{3-36} concentrations were independently and positively associated with type 2 diabetes and obesity-associated insulin resistance.[15] Furthermore, in this study, PYY_{3-36} concentrations were associated with adiposity,[15] in contrast to a number of other studies showing that low PYY_{3-36} concentrations are associated with adiposity.[10,19] Notably, first-degree relatives of subjects with type 2 diabetes exhibited lower fasting PYY concentrations than matched controls, but in this study, only total PYY was measured.[20] Ukkola et al. studying patients with coronary artery disease additionally found an association between high fasting PYY_{3-36} concentrations and fasting glucose concentrations, although the association was abolished after adjustment for BMI.[15]

The authors speculated that differences in age, other population's characteristics, and different experimental setting may explain these divergent results.[15] Furthermore, disturbed PYY concentrations may be a cause or a consequence of type 2 diabetes and associated obesity in cross-sectional studies. Thus, associations shown may be dependent on the stage of diabetes or obesity. Our findings are consistent with observations in subjects with extreme obesity (mean BMI, 53), examined after a fat load.[21] Insulin resistance impaired the PYY response to a fat load, but the study did not find significant differences in PYY levels between subjects with different degrees of insulin resistance or with diabetes in these morbidly obese subjects.[21] Unfortunately, in this study, only total PYY was measured.

While some authors have speculated that a state of partial PYY resistance may be present in patients with coronary artery disease, parallel to insulin and leptin resistance,[15] we found no relationship between circulating PYY_{3-36} and leptin or insulin concentrations. Obesity and insulin resistance are characterized by subclinical inflammation, and markers of inflammation associate both with visceral obesity and insulin resistance.[1] In this study, fasting PYY_{3-36} concentrations were not related to markers of inflammation, including CRP, ferritin, and white cell count.

The association between PYY_{3-36} concentrations and systolic blood pressure appears to be a novel one, and underscores a possible avenue of future research. The relationship was observed both in the entire sample, and after exclusion of subjects taking antihypertensive medication. PYY appears to induce vasoconstriction by Y1R agonism and has been shown to be present in atherosclerotic plaque in animal studies.[22] However, the PYY_{3-36} metabolite that binds to Y2R neurons has not been previously associated with increased blood pressure to our knowledge, although variants in the Y2R genes are associated with a reduced risk of hypertension.[23] PYY acts as a vasoconstricting agent and PYY infusion cause significant vasoconstricting effects.[24] Divergent effects of PYY_{1-36} and PYY_{3-36} on appetite and glucose homeostasis may or may not be paralleled by divergence in regard to blood pressure. Among patients with coronary artery disease, no relationship was observed between PYY_{3-36} and blood pressure.[15] However, subjects with extreme obesity may display a disrupted PYY_{3-36} function. Further study will be needed to clarify this finding.

Limitations

The main study limitation is that we did not have postprandial PYY_{3-36} concentrations. It also would have been

useful to compare the results to those of a lean control group or to individuals with lesser obesity, but these data are not available. Furthermore, novel markers of subclinical inflammation such as cytokine concentrations were not measured. Blood pressure measurement was based on a clinical measurement on a single occasion. A comparison with ambulatory 24-hour blood pressure would have been informative, but we did not have these measurements.

Conclusion

$PYY_{3\text{-}36}$ appears to be linked to systolic blood pressure in extremely obese individuals, but not associated to other components of MetSyn, insulin resistance, inflammation, or leptin. These findings suggest the need to distinguish pathways whereby $PYY_{3\text{-}36}$ may influence cardiovascular risk factors.

Author Disclosure Statement

No conflicting financial interests exist

References

1. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005;365:1415–1428.
2. Aguilar M, Bhuket T, Torres S, et al. Prevalence of the metabolic syndrome in the United States, 2003–2012. *JAMA* 2015;313:1973–1974.
3. Barquera S, Pedroza-Tobías A, Medina C, et al. Global overview of the epidemiology of atherosclerotic cardiovascular disease. *Arch Med Res* 2015;46:328–338.
4. Sturm R, Hattori A. Morbid obesity rates continue to rise rapidly in the United States. *Int J Obes* 2013;37:889–891.
5. Tonstad S, Després J-P. Treatment of lipid disorders in obesity. *Expert Rev Cardiovasc Ther* 2011;9:1069–1080.
6. Tinahones FJ, Garrido-Sánchez L, Murri M, et al. Particular characteristics of the metabolic syndrome in patients with morbid obesity. *Endocrinol Nutr* 2013;60:127–135.
7. Ledoux S, Coupaye M, Essig M, et al. Traditional anthropometric parameters still predict metabolic disorders in women with severe obesity. *Obesity* 2010;18:1026–1032.
8. Vinciguerra F, Baratta R, Farina MG, et al. Very severely obese patients have a high prevalence of type 2 diabetes mellitus and cardiovascular disease. *Acta Diabetol* 2013; 50:443–449.
9. Hussain SS, Bloom SR. The regulation of food intake by the gut-brain axis: Implications for obesity. *Int J Obes* 2013; 37:625–633.
10. Manning S, Batterham RL. The role of gut hormone peptide YY in energy and glucose homeostasis: Twelve years on. *Annu Rev Physiol* 2014;76:585–608.
11. Price SL, Bloom SR. Protein PYY and its role in metabolism. Delhanty PJD, van der Lely AJ (eds): How gut and brain control metabolism. *Front Horm Res Basel Karger* 2014;42:147–154.
12. Cahill F, Ji Y, Wadden D, et al. The association of serum total peptide YY (PYY) with obesity and body fat measures in the CODING study. *PLoS One* 2014;9:e95235.
13. Batterham RL, Cohen MA, Ellis SM, et al. Inhibition of food intake in obese subjects by peptide $YY_{3\text{–}36}$. *N Engl J Med* 2003;349:941–948.
14. Zac-Varghese S, De Silva A, Bloom SR. Translational studies on PYY as a novel target in obesity. *Curr Opin Pharmacol* 2011;11:582–585.
15. Ukkola OH, Puurunen V-P, Piira O-P, et al. High serum fasting peptide YY (3–36) is associated with obesity-associated insulin resistance and type 2 diabetes. *Regul Pept* 2011;170:38–42.
16. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004;27:1487–1495.
17. Shih PB, Wang L, Chiron S, et al. Peptide YY (PYY) gene polymorphisms in the 3′-untranslated and proximal promoter regions regulate cellular gene expression and PYY secretion and metabolic syndrome traits in vivo. *J Clin Endocrinol Metab* 2009;94:4557–4566.
18. Chandarana K, Gelegen C, Irvine EE, et al. Peripheral activation of the Y2-receptor promotes secretion of GLP-1 and improves glucose tolerance. *Mol Metab* 2013;2:142–152.
19. Zwirska-Korczala K, Konturek SJ, Sodowski M, et al. Basal and postprandial plasma levels of PYY, ghrelin, cholecystokinin, gastrin and insulin in women with moderate and morbid obesity and metabolic syndrome. *J Physiol Pharmacol* 2007;58 Suppl 1:13–35.
20. Boey D, Heilbronn L, Sainsbury A, et al. Low serum PYY is linked to insulin resistance in first-degree relatives of subjects with type 2 diabetes. *Neuropeptides* 2006;40: 317–324.
21. Fernández-Garcia JC, Murri M, Coin-Aragüez L, et al. GLP-1 and peptide YY secretory response after fat load is impaired by insulin resistance, impaired fasting glucose and type 2 diabetes in morbidly obese subjects. *Clin Endocrinol* 2014;80:671–676.
22. Smith RM, Klein R, Kruzliak P, et al. Role of peptide YY in blood vessel function and atherosclerosis in a rabbit model. *Clin Exper Pharmacol Physiol* 2015;42:648–652.
23. Santoro N, del Giudice EM, Grandone A, et al. Y2 receptor gene variants reduce the risk of hypertension in obese children and adolescents. *J Hypertens* 2008;26:1590–1594.
24. Adrian TE, Sagor GR, Savage AP, et al. Peptide YY kinetics and effects on blood pressure and circulating pancreatic and gastrointestinal hormones and metabolties in man. *J Clin Endocrinol Metab* 1986;63:803–807.

Address correspondence to:
Sasa Dusanov, MD
Section for Preventive Cardiology
Department of Endocrinology
Morbid Obesity and Preventive Medicine
Oslo University Hospital
P.b. 4956 Nydalen
Oslo N-0424
Norway

E-mail: sasdus@ous-hf.no

METABOLIC SYNDROME AND RELATED DISORDERS
Volume 14, Number 10, 2016

Pp. 500–506
DOI: 10.1089/met.2016.0062

Characteristics of Metabolic Syndrome in Morbidly Obese Subjects

Sasa Dusanov, MD, Eli Heggen, MD, and Serena Tonstad, MD, PhD

Abstract

Background: Metabolic syndrome (MetSyn) magnifies risks of cardiovascular disease (CVD) and type 2 diabetes, but its expression varies within the obese population. We examined body mass index (BMI), metabolic traits, and fat distribution in morbidly obese individuals.

Methods: Lipids and inflammatory, oxidative stress and hepatic biomarkers in 346 women and 203 men (BMI $\geq 35\,kg/m^2$ and co-morbidity or $\geq 40\,kg/m^2$) were stratified by MetSyn components (1–5, excluding diabetes). Age- and smoking-adjusted partial correlations were calculated. Dual-energy X-ray absorptiometry was measured in 206 participants.

Results: Apolipoprotein B, ferritin, uric acid, and alanine aminotransferase (ALT) concentrations worsened with increasing MetSyn components ($P \leq 0.0001$), while BMI and LDL-cholesterol showed no association. BMI correlated inversely with triglycerides ($r = -0.16$, $P = 0.03$) and positively with HDL-cholesterol in men ($r = 0.16$, $P = 0.02$), but not in women. BMI correlated with C-reactive protein (CRP) ($r = 0.32$, $P < 0.0001$; $r = 0.24$, $P < 0.0001$ in men and women, respectively) and white blood cell count ($r = 0.24$, $P = 0.001$ in men; $r = 0.15$, $P = 0.008$ in women). Truncal fat percentage correlated to CRP ($r = 0.31$, $P = 0.03$; $r = 0.20$, $P = 0.02$ in men and women, respectively). In women, number of MetSyn components was inversely related to truncal and peripheral fat ($r = -0.20$, $P = 0.02$; $r = -0.42$, $P < 0.0001$, respectively) as was ALT ($r = -0.21$, $P = 0.009$; $r = -0.38$, $P < 0.0001$, respectively) and triglycerides with peripheral fat ($r = -0.38$, $P < 0.0001$), while HDL cholesterol was positively associated with truncal and peripheral fat ($r = 0.26$; $P = 0.001$).

Conclusions: BMI and fat distribution showed expected associations to inflammation biomarkers, but paradoxical relations between fat indices, and MetSyn components and biomarkers were seen. This suggests a need for better markers of CVD risk in morbid obesity.

Introduction

Obesity represents one of the most complex public health challenges for our societies. Cardiometabolic disorders (metabolic syndrome [MetSyn] and type 2 diabetes) follow in the wake of obesity adding to health burdens on the population. Of all diseases caused by obesity, relative risks of type 2 diabetes are the highest.[1] Type 2 diabetes is characterized by insulin resistance that is tightly related to abdominal obesity and is accompanied by changes in metabolic traits and subclinical inflammation that lead to cardiovascular disease (CVD).[2]

Clinically the presence of three or more of five risk factors, namely abdominal obesity, low HDL cholesterol, high triglycerides, high fasting glucose, and high blood pressure characterizes MetSyn.[3]

Persons with morbid obesity, defined as body mass index (BMI) $\geq 35\,kg/m^2$ with co-morbidities or $\geq 40\,kg/m^2$ regardless of co-morbidities, typically carry increased burdens of psychological and physical discomfort and disease. While the risk of CVD is clearly magnified by the presence of MetSyn or type 2 diabetes, CVD risk factors and metabolic traits may not increase linearly with increasing BMI and may differ by gender.[4] While lipid disturbances are common, they are not uniformly related to total body fat mass.[5] Subgroups of morbid obesity have been identified, which appear metabolically healthy and exhibit normal lipids or high insulin sensitivity.[6,7] Furthermore, waist circumference is uniformly above standard cutoff levels that are used to define MetSyn and thus of lesser utility in determining risks in the morbidly obese population.

The purpose of the study was to examine the relationships of MetSyn components and CVD risk factors to BMI and fat

Section for Preventive Cardiology, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway.

distribution in morbidly obese men and women. We examined subjects with morbid obesity grouped according to their degree of metabolic disturbance, with the goal of better understanding the expression and utility of CV risk markers in this population.

Materials and Methods

Patients referred consecutively to the Preventive Cardiology Clinic at Oslo University Hospital, Oslo, Norway, participated between April 2005 and December 2010. The study conformed to the Helsinki Declaration and was evaluated by the Ethics committee for region 1 in Norway.

Participants with BMI $\geq 35\,kg/m^2$ and co-morbidity, or related disorders (including hypertension, sleep apnea, dyspnea, polycystic ovarian syndrome, asthma, hypercholesterolemia, gout, musculoskeletal symptoms, gall bladder symptoms, esophageal reflux, pulmonary or deep vein embolism, intermittent claudication, angina pectoris, depression, or eating disorder) or BMI $\geq 40\,kg/m^2$ regardless of co-morbidity, but without diabetes were included ($n = 549$). Subjects with type 2 diabetes ($n = 209$) were not included in current analyses.

After written informed consent, participants completed a health questionnaire and underwent anthropometric measurements. A constant tension body tape measure was used to determine waist and hip circumferences. Waist circumference was measured at midpoint between the inferior costal margin and the highest point of the iliac crest, and hip circumference was measured at the widest point around the hips. Height was measured using a stadiometer and recorded to the nearest cm. Patients were weighed to the nearest 1.0 kg using a calibrated mobile electronic scale (Seca 720; Medical Scales and Measuring Systems). BMI was calculated in accordance to the Quetelet's formula: Body weight in kilograms divided by the square of body height in meters (kg/m^2). Body total and regional fat percentages were analyzed by using dual X-ray absorptiometry (DXA) (Lunar DPX-L, Lunar) in 206 individuals. Only individuals with body weights below 140 kg could be examined by DXA. Blood pressure was measured automatically using an automatic blood pressure monitor (52000 Series Vital Signs Monitor; Welch Ally) following a 5-min rest.

Subjects were stratified by number of MetSyn components (from 1 to 5). Definition of MetSyn components was as follows: waist circumference ≥ 102 cm for men and ≥ 88 cm for women; systolic blood pressure ≥ 130 mmHg and/or diastolic ≥ 85 mmHg, or drug treatment for hypertension; triglycerides ≥ 1.7 mM; HDL-cholesterol ≤ 1.0 for men or ≤ 1.3 for women; and fasting glucose ≥ 5.6 mM.

Laboratory analyses

Participants were instructed to fast overnight for at least 10 hr, before providing blood samples between 8:00 and 11:00 a.m. Analyses of blood samples were performed at Oslo University Hospital (Clinical Chemistry Laboratory at Ullevål and Endocrine Laboratory at Aker). Total cholesterol, HDL-cholesterol, triglycerides, glucose, alanine aminotransferase (ALT), uric acid, creatinine, and high-sensitivity C-reactive protein (CRP) concentrations were measured on an automated analyzer Cobas Integra 800 (Roche Diagnostics). LDL-cholesterol was calculated using Friedewald's formula. Apolipoprotein B and lipoprotein (a) were determined with an immunoturbidimetric assay on an automated analyzer (Cobas Tinaquant 917, Roche/Hitachi; Roche Diagnostics). White blood cells were analyzed using Sysmex XE 2100 (Sysmex). Serum ferritin was determined by an ADIVA Centaur analysis (ADIVA Centaur; Siemens Healthcare Diagnostics, Inc.).

Statistical analyses

Statistical analyses were performed using SPSS 21 (SPSS, Inc.). Categorical and continuous data are presented as counts and percentages, or mean ± standard deviation (SD), respectively. Variables were tested for normality and logarithmically transformed values for lipoprotein (a), CRP, ferritin, white blood cell count, and ALT were used where indicated. One-way analysis of variance (ANOVA) was performed to compare components of MetSyn, other lipids, and inflammatory biomarkers. Partial correlation coefficients (corrected for age and smoking) were calculated to analyze correlations between BMI and waist circumference and MetSyn components, other lipids, and inflammatory biomarkers. Two-sided P values of <0.05 were considered statistically significant.

Results

A total of 346 women and 203 men aged between 18 and 78 years and BMI between $35\,kg/m^2$ and $74\,kg/m^2$ participated. Sample characteristics and MetSyn components are presented in Table 1. Two-thirds (373 of 547) met criteria of MetSyn (three or more components). Age and the proportion of males increased with increasing numbers of components of MetSyn, and the level of each component increased with increasing numbers of components. BMI was not associated with number of MetSyn components. As shown in Table 2, there was no relationship between LDL-cholesterol concentrations and MetSyn components, while Lp(a) concentrations decreased as the number of components increased. Total cholesterol, apolipoprotein B, ferritin, uric acid, and ALT levels increased as number of components of MetSyn increased.

BMI and waist circumference were highly correlated ($r = 0.67$, $P < 0.0001$ in women; $r = 0.85$, $P < 0.0001$ in men). Partial correlation coefficients between BMI and waist circumference and metabolic traits and biomarkers are shown in Table 3. Number of MetSyn components did not correlate with BMI, and weakly with waist circumference (only in women). In men, all components of MetSyn correlated with BMI, with the exception of fasting glucose. However, waist circumference correlated only with blood pressure. The correlations of HDL-cholesterol and triglycerides to BMI and waist circumference were opposite to expected (positively for HDL-cholesterol and inversely for triglycerides). In women, only systolic blood pressure correlated with BMI and waist circumference.

CRP concentrations and white blood cell count correlated with both BMI and waist circumference in both genders. Uric acid concentrations correlated with BMI, while ALT correlated with waist circumference in women.

The mean (SD) BMI of the group with DXA measurements was 40.5 (3.6) kg/m^2, versus 43.4 (5.9) kg/m^2 in the group that did not take part in DXA ($P < 0.0001$). As was the case in the total sample, over two-thirds had three or more criteria of MetSyn (142 of 206). As shown in Table 4,

TABLE 1. AGE, GENDER, ANTHROPOMETRICS, SMOKING STATUS, COMPONENTS OF METABOLIC SYNDROME, AND MEDICATION USE STRATIFIED BY NUMBER OF METABOLIC SYNDROME COMPONENTS (N=547)

	Number of components					
Parameter	*One* n=*43*	*Two* n=*131*	*Three* n=*200*	*Four* n=*121*	*Five* n=*52*	P
Age, years	33.5 (11.6)	39.9 (12.9)	40.6 (12.5)	40.6 (11.2)	44.9 (9.6)	<0.0001
Male, *n* (percentage)	6 (14.0)	42 (32.1)	74 (37.0)	54 (44.6)	27 (51.9)	0.001
BMI, kg/m^2	42.1 (5.1)	42.5 (5.3)	42.4 (5.4)	42.3 (5.4)	41.6 (5.2)	0.88
Smokers, *n* (percentage)	13 (30.2)	25 (19.1)	45 (22.5)	31 (25.6)	17 (32.7)	0.26
Metabolic syndrome components						
Waist circumference, cm	116.5 (11.6)	122.6 (14.6)	124.9 (15.0)	127.0 (14.3)	125.3 (14.0)	0.001
Systolic BP, mm Hg	117 (8)	130 (14)	135 (18)	135 (14)	138 (15)	<0.0001
Diastolic BP, mmHg	77 (6)	83 (9)	86 (11)	88 (10)	90 (10)	<0.0001
HDL-cholesterol, mM	1.5 (0.3)	1.3 (0.3)	1.2 (0.3)	1.1 (0.2)	0.9 (0.2)	<0.0001
Triglycerides, mM	1.1 (0.3)	1.2 (0.4)	1.8 (1.4)	2.4 (1.1)	3.2 (1.6)	<0.0001
Glucose, mM	4.9 (0.3)	5.1 (0,4)	5.4 (0.7)	5.5 (0.6)	6.0 (0.4)	<0.0001
Use of statins (percentage)	0 (0)	9 (6.9)	22 (11.0)	13 (10.7)	6 (11.5)	0.15
Use of antihypertensive drugs (percentage)	0 (0)	27 (20.6)	57 (28.5)	42 (34.7)	21 (40.4)	<0.0001

Mean (SD) shown except for percentages. *P* value indicates ANOVA comparison across groups.
BMI, body mass index; BP, blood pressure.

number of MetSyn components in men and women was inversely associated with fat percentages of total body mass and truncal region, and also inversely associated with fat percentage of peripheral region in women. In Table 5, age- and smoking-adjusted partial correlations between MetSyn components, other lipids, and biomarkers with fat percentage of total body mass and truncal and peripheral fat are shown. In men, CRP concentrations correlated with increasing fat percentage of total body mass and central fat. These relationships were also seen in women. However, in women, paradoxical relationships between number of MetSyn components and HDL-cholesterol and triglyceride concentrations to total fat percentage and to peripheral fat were seen (lesser number of components and lower triglycerides, but higher HDL cholesterol with increasing fat). HDL-cholesterol correlated positively to truncal fat percentage. Also, ALT concentrations were inversely associated with all fat percentages in women, and uric acid was inversely associated with total and peripheral fat percentages.

Discussion

Our main findings were that degree of obesity, as measured by BMI or waist circumference, did not predict MetSyn components, with the exception of systolic blood pressure in women with morbid obesity. In men with morbid obesity, systolic and diastolic blood pressures were positively related to BMI and waist circumference, but paradoxical relationships between BMI and lipids were observed. Furthermore, increasing number of components of MetSyn was associated with decreasing total and truncal fat in women and men and decreasing peripheral fat in women. In women, paradoxical relationships were seen between total, truncal,

TABLE 2. OTHER LIPIDS AND LIPOPROTEINS, BIOMARKERS OF INFLAMMATION, AND OTHER MARKERS OF METABOLIC SYNDROME STRATIFIED BY NUMBER OF METABOLIC SYNDROME COMPONENTS (N=547)

	Number of components					
Parameter	*One* n=*43*	*Two* n=*131*	*Three* n=*200*	*Four* n=*121*	*Five* n=*52*	P[a]
Other lipids						
Total cholesterol, mM	5.2 (1.1)	5.0 (0.9)	5.3 (1.2)	5.5 (1.1)	5.4 (0.9)	0.009
LDL-cholesterol, mM	3.2 (1.0)	3.2 (1.2)	3.3 (1.0)	3.4 (0.9)	3.1 (1.0)	0.56
Lipoprotein (a), nM	319 (122, 782)	222 (84, 508)	144 (63, 354)	180 (87, 471)	161 (63, 280)	0.03
Apolipoprotein B, g/L	0.9 (0.2)	0.9 (0.2)	1.0 (0.2)	1.1 (0.2)	1.1 (0.2)	<0.0001
Biomarkers						
C-reactive protein, mg/L	8.5 (4.0, 16.0)	6.0 (3.0, 11.0)	6.0 (3.0, 11.0)	6.0 (3.3, 10.8)	5.0 (3.0, 8.0)	0.09
White blood cells, $\times 10^9$/L	6.7 (5.6, 8.3)	6.5 (5.4, 8.1)	6.8 (5.8, 8.0)	6.8 (5.8, 7.9)	6.9 (5.8, 8.2)	0.24
Ferritin, μg/L	54 (38, 102)	73 (36, 150)	92 (30, 150)	118 (51, 211)	162 (77, 264)	<0.0001
Uric acid, μM	336 (74)	363 (75)	385 (91)	405 (81)	431 (85)	<0.0001
ALT, U/L	20 (17, 31)	30 (20, 47)	31 (22, 45)	34 (24, 47)	36 (24, 57)	<0.0001

Mean (SD) shown except for lipoprotein (a), C-reactive protein (CRP), ferritin, white blood cells, and alanine aminotransferase (ALT) for which median (25th, 75th percentiles) are shown.
For total cholesterol, 1 missing; for LDL-cholesterol, 25 missing (could not be estimated because high level of triglycerides); for lipoprotein (a), 12 missing; for apolipoprotein B, 14 missing; for CRP, 10 missing; for ferritin, 14 missing; for white blood cells, 18 missing; for uric acid, 13 missing; and for ALT, 12 missing.
[a]*P* value indicates ANOVA comparison across groups.

TABLE 3. BMI AND WAIST CIRCUMFERENCE CORRELATIONS WITH NUMBER OF METABOLIC SYNDROME COMPONENTS, COMPONENTS OF METABOLIC SYNDROME, OTHER LIPIDS AND LIPOPROTEINS, BIOMARKERS OF INFLAMMATION, AND OTHER MARKERS OF METABOLIC SYNDROME, ADJUSTED FOR AGE AND SMOKING STATUS (*N*=547)

	Male (n=*203*)				*Female* (n=*344*)			
	BMI		*Waist circumference*		*BMI*		*Waist circumference*	
	Coefficient	P	*Coefficient*	P	*Coefficient*	P	*Coefficient*	P
#MetSyn components	–0.07	0.34	0.01	0.88	0.05	0.38	0.11	0.04
Systolic BP (all)	0.27	<0.0001	0.29	<0.0001	0.19	<0.0001	0.19	<0.0001
Diastolic BP (all)	0.25	<0.0001	0.32	<0.0001	0.01	0.79	0.05	0.34
Systolic BP (no medication)	0.32	<0.0001	0.35	<0.0001	0.21	<0.0001	0.23	<0.0001
Diastolic BP (no medication)	0.25	0.004	0.29	<0.0001	0.05	0.46	0.09	0.14
HDL-cholesterol	0.16	0.02	0.08	0.24	0.02	0.69	0.01	0.83
Triglycerides	–0.16	0.03	–0.11	0.12	–0.02	0.71	0.03	0.59
Glucose	0.13	0.06	0.07	0.34	0.02	0.79	0.07	0.21
Total cholesterol	0.07	0.33	0.01	0.89	–0.06	0.24	0.03	0.56
LDL-cholesterol	0.13	0.07	0.04	0.60	–0.04	0.44	0.04	0.45
Lipoprotein (a)	–0.05	0.45	–0.08	0.29	–0.07	0.20	–0.10	0.07
Apolipoprotein B	0.11	0.14	0.07	0.30	–0.06	0.30	0.03	0.59
C-reactive protein	0.32	<0.0001	0.27	<0.0001	0.24	<0.0001	0.24	<0.0001
White blood cells	0.24	0.001	0.20	0.005	0.15	0.008	0.19	0.001
Ferritin	0.03	0.72	0.09	0.21	–0.01	0.83	0.04	0.49
Uric acid	0.04	0.57	0.02	0.75	0.19	<0.0001	0.10	0.07
ALT	0.05	0.47	0.06	0.38	0.07	0.24	0.14	0.01

and peripheral fat percentages and HDL-cholesterol, triglyceride, and ALT concentrations. Positive associations between BMI, waist circumferences, fat percentages, and inflammatory biomarkers were noted. Associations were controlled for age and smoking, given the effects of smoking on MetSyn.[8]

The strength of this study is the inclusion of a large sample of consecutive patients of which over one-third consisted of men. Notably, there was no association between BMI and number of MetSyn components, suggesting that other factors than BMI determine MetSyn in morbid obesity, as suggested previously.[9] Contrastingly, some studies have reported that metabolic traits remain associated with traditional anthropometric measures in severe obesity;[10–12] however, these studies have included limited samples of severely obese individuals. Furthermore, we found only a weak association between waist circumference and number of MetSyn components in women and none in men. This is in line with a previous study of 100 morbidly obese individuals, in which waist circumference did not correlate with the prevalence of MetSyn, its severity, or with visceral fat area measured by CT.[9] However, CT-assessed visceral fat did show an association with MetSyn in this study.[9] These findings put together suggest that waist circumference is less useful as a tool to assess MetSyn in the morbidly obese than in nonmorbidly obese populations, and more specific tools may be needed, including visualization by CT or other methods.

Of our total sample, 68% met the harmonized definition of MetSyn, while the remaining one-third may be characterized as metabolically healthy with high waist circumference, but exhibiting none or only one other MetSyn component above the proposed cutoff levels. This proportion is similarly to that previously reported in morbidly obese individuals.[7–9] The metabolically healthy participants were more likely to be female and were younger than those exhibiting a larger number of components of MetSyn. The predominance of younger individuals and women is in line with the notion that metabolically healthy obese individuals are unlikely to be permanently protected from metabolic disturbances.[6] Furthermore, compared with metabolically

TABLE 4. FAT DISTRIBUTION STRATIFIED BY NUMBER OF METABOLIC SYNDROME COMPONENTS (*N*=206)

	Number of components					
Parameter	*One* n=*14*	*Two* n=*50*	*Three* n=*77*	*Four* n=*43*	*Five* n=*22*	P[a]
Men	*n*=0	*n*=10	*n*=20	*n*=12	*n*=10	
Fat percentage of total body mass	—	44.3 (3.3)	39.9 (3.2)	41.5 (3.7)	41.2 (4.0)	0.02
Fat percentage of truncal region	—	54.6 (2.4)	50.6 (3.8)	52.0 (4.0)	49.9 (4.5)	0.02
Fat percentage of peripheral region	—	45.8 (4.8)	41.4 (4.9)	42.4 (4.6)	42.8 (5.5)	0.16
Women	*n*=14	*n*=40	*n*=57	*n*=31	*n*=12	
Fat percentage of total body mass	54.3 (2.9)	53.7 (3.0)	51.2 (3.4)	50.9 (4.2)	49.5 (3.6)	<0.0001
Fat percentage of truncal region	59.3 (2.2)	59.1 (4.3)	57.2 (4.0)	58.3 (4.4)	55.5 (4.0)	0.03
Fat percentage of peripheral region	57.8 (4.1)	57.6 (3.7)	55.2 (3.8)	53.4 (4.6)	52.3 (3.5)	<0.0001

Mean (SD) shown.
[a]*P* indicates ANOVA comparison across groups.

Table 5. Partial Correlations Between Total, Truncal, and Peripheral Fat Percentages in Men and Women with Components of Metabolic Syndrome, Other Lipids and Lipoproteins, and Biomarkers Adjusted for Age and Smoking ($N=206$)[a]

	Men						*Women*					
	Total		*Truncal*		*Peripheral*		*Total*		*Truncal*		*Peripheral*	
	r	P	r	P	r	P	r	P	r	P	r	P
#MetSyn Components	−0.13	0.37	−0.19	0.18	−0.01	0.54	−0.38	<0.0001	−0.20	0.02	−0.42	<0.0001
Metabolic syndrome components												
Systolic BP	−0.24	0.10	−0.19	0.18	−0.23	0.12	−0.15	0.06	−0.08	0.35	−0.10	0.24
Diastolic BP	−0.14	0.32	−0.03	0.85	−0.24	0.10	−0.13	0.13	−0.11	0.18	−0.10	0.25
Systolic BP (no meds)	−0.26	0.18	−0.31	0.10	−0.24	0.21	−0.16	0.10	−0.02	0.87	−0.12	0.22
Diastolic BP (no meds)	−0.18	0.34	−0.14	0.47	−0.30	0.12	−0.18	0.06	−0.13	0.16	−0.17	0.07
HDL-cholesterol	−0.02	0.90	0.01	0.97	0.02	0.87	0.31	<0.0001	0.26	0.001	0.26	0.001
Triglycerides	−0.17	0.24	−0.17	0.24	−0.21	0.15	−0.31	<0.0001	−0.13	0.10	−0.38	<0.0001
Glucose	−0.02	0.88	−0.03	0.85	−0.01	0.94	−0.11	0.19	−0.03	0.73	−0.12	0.13
Other lipids and lipoproteins												
Total cholesterol	0.03	0.83	0.01	0.96	0.02	0.91	−0.04	0.63	0.06	0.47	−0.10	0.21
LDL-cholesterol	0.15	0.34	0.08	0.61	0.12	0.44	−0.03	0.69	0.02	0.78	−0.06	0.48
Lipoprotein (a)	0.15	0.30	0.15	0.31	0.08	0.59	0.12	0.16	0.06	0.45	0.10	0.21
Apolipoprotein B	0.07	0.64	0.02	0.88	−0.02	0.89	−0.15	0.08	−0.06	0.48	−0.19	0.02
Biomarkers												
C-reactive protein	0.42	0.003	0.31	0.03	0.27	0.06	0.28	0.001	0.20	0.02	0.27	0.001
White blood cell count	0.10	0.49	0.13	0.36	−0.04	0.79	−0.06	0.48	−0.03	0.73	−0.08	0.34
Ferritin	0.09	0.55	−0.01	0.97	0.11	0.44	−0.11	0.20	0.04	0.66	−0.14	0.08
Uric acid	−0.08	0.57	−0.20	0.18	0.01	0.95	−0.20	0.01	−0.07	0.42	−0.23	0.006
ALT	−0.10	0.48	−0.14	0.33	−0.15	0.31	−0.37	<0.0001	−0.21	0.009	−0.38	<0.0001

r = partial correlation coefficient.
[a]For LDL-cholesterol, 8 missing (could not be estimated because high level of triglycerides); for Lp(a), 2 missing; for apolipoprotein B, 3 missing; and for C-reactive protein, 3 missing.

healthy normal weight individuals, obese persons are at an increased risk for adverse outcomes, even in the absence of metabolic abnormalities.[13]

A key indicator of metabolic risks of obesity is the presence of subclinical inflammation, as evidenced by an elevated high-sensitivity CRP concentration, and increased concentrations of other inflammatory markers, including white blood cell count.[14,15] Elevated CRP is prevalent even among individuals with metabolically healthy obesity,[14] and may underlie the risk of adverse outcomes in this group.[13] We observed markedly elevated high-sensitivity CRP concentrations across all metabolic risk categories, and CRP concentrations were correlated with BMI and waist circumference. Likewise, white blood cell counts, although not grossly elevated, correlated with BMI and waist circumference in both genders. While these biomarkers of inflammation did not associate with severity of MetSyn, as indicated by the number of components, CRP concentrations showed statistically significant associations with all fat depots, while white blood cell count showed no associations.

Other biomarker indicators of obesity-related stress include oxidative stress and liver dysfunction.[15] Both ferritin and uric acid concentrations were associated with severity of MetSyn, as seen in many obese populations,[15] suggesting that they may be useful markers of metabolic damage in the morbidly obese, although causality cannot be determined in a cross-sectional study. These biomarkers were not associated with waist circumference. Uric acid concentrations were positively associated with BMI only in women, but a novel finding was the inverse relation between uric acid in relation to total and peripheral fat depots in women. While uric acid acts as an antioxidant in response to increased oxidative stress in normal weight individuals, it does not act as an antioxidant in patients with MetSyn, as the system appears to be saturated and unable to respond appropriately to increased oxidative stress.[16]

ALT concentrations indicating hepatic dysfunction were classically associated with severity of MetSyn and waist circumference in women. This finding is in line with a study of liver fat in morbidly obese patients where MetSyn was found to be independently related to fatty liver measured by CT.[9] However, inverse associations were found between ALT and total, truncal, and peripheral fat in women, a surprising finding that questions the suitability of DXA in the extremely obese.

One of the best established links between obesity and CVD risk is obesity-related dyslipidemia,[5] which is characterized by reduced lipolysis of triglyceride-rich lipoproteins, and the development of small dense LDL particles, small dense HDL particles, and relatively high apolipoprotein B levels. Notably, these changes do not appear to depend on total body fat mass.[5] Previously, studies have revealed lower free fatty acids and triglyceride concentrations before and after fat overloads in patients with MetSyn with or without morbid obesity.[16] In line with this, we found no relationship between BMI or waist circumference and lipids in women, and in men, relationships were paradoxical, with positive relations between BMI and HDL-cholesterol and an inverse relationship between BMI and triglycerides. We suggest that divergent findings regarding risk factor expression may be due to the sequestering of fat in adipose tissue in morbid obesity. In elegant experiments, Tinahones et al. showed that in the face

of increased lipolysis, the net release of free fatty acids into the bloodstream is counteracted by increased reuptake and cycling in the morbidly obese.[17] This is facilitated by increased expression of PPARγ and other enzymes involved in lipid storage, thus ensuring the storage and relocalization of the excess triglyceride.[17]

A trend toward lower lipoprotein (a) concentrations with increasing number of MetSyn components was noted in our study. This observation has been made previously in a predominantly healthy Asian occupational cohort and appeared to be independent of confounders.[18] Also, in a cohort of Italian hypertensive patients, lipoprotein (a) levels were significantly, independently, and progressively lower with increasing insulin resistance.[19] Lipoprotein (a) concentrations are strongly linked to apolipoprotein (a) allele size and expression, while dietary, lifestyle, and environmental factors have less influence. Thus, these data suggest that shared genetic mechanisms may underlie the associations, although other nongenetic explanations are possible.

In the subgroup that completed the DXA procedure, severity of MetSyn appeared to be associated with lower total, truncal, and peripheral fat depots (Table 4). Given that BMI did not differ according to MetSyn severity, this finding underscores the role of large depots in ''absorbing'' metabolic risks. The protective associations between lower body fat and cardiovascular risk in nonobese populations have been long known.[20] We previously reported that leg fat mass protected against metabolic risks in obese women, although not in obese men.[21] A study of severely obese premenopausal women, with BMI >40 found that leg fat correlated negatively with cardiovascular risk factors.[22] These findings extend these observations to women and men with morbid obesity and suggest that adequate fat depots may be advantageous, although ectopic fat would not be expected to protect against metabolic risks even in this population. This idea would need in depth and further investigation with CT- or MR-based methodology. Furthermore, HDL-cholesterol concentrations were positively associated with all fat depots, and triglyceride concentrations were inversely associated with total and peripheral depots in women. Notably, a large study of South Koreans found that metabolically healthy obese persons had an attenuated risk of preclinical atherosclerosis in contrast to metabolically abnormal obese and nonobese persons.[23] While not directly comparable to the current findings, this suggests that obesity in itself is less important than the metabolic abnormalities.

Study limitations

We lacked data on dietary patterns, alcohol consumption, and physical activity of the participants. Only 38% participated in the DXA due, in part, to limitations in performing the procedure in very heavy individuals. Thus, the findings regarding fat distribution may not apply to individuals with extreme obesity. Furthermore, hepatic fat was not quantified through ultrasound or other methods. We did not adjust for use of statins, but findings excluding users of statins did not differ substantially from shown associations (data not shown).

Conclusion

MetSyn severity does not seem to linearly increase with BMI, suggesting a need for better markers of CVD risk in the morbidly obese, particularly women. The paradoxical inverse correlation between BMI and fat distribution with lipids and other biomarkers suggests protective effects of fat depots in the morbidly obese, but prospective studies are needed to establish causality.

Author Disclosure Statement

No competing financial interests exist. All authors agree with the content of the text and tables.

References

1. Knight JA. Diseases and disorders associated with excess body weight. *Ann Clin Lab Sci* 2011;41:107–121.
2. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005;365:1415–1428.
3. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute. American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009;120:1640–1645.
4. Morais AA, Faintuch J, Leal AAD, et al. Inflammation and biochemical features of bariatric candidates: Does gender matter? *Obes Surg* 2011;21:71–77.
5. Klop B, Elte JWF, Cabezas MC. Dyslipidemia in obesity: Mechanisms and potential targets. *Nutrients* 2013;5:1218–1240.
6. Plourde G, Karelis AD. Current issues in the identification and treatment of metabolically healthy but obese individuals. *Nutr Metab Cardiovasc Dis* 2014;24:455–459.
7. Soverini V, Moscatiello S, Villanova N, et al. Metabolic syndrome and insulin resistance in subjects with morbid obesity. *Obes Surg* 2010;20:295–301.
8. Sun K, Liu J, Ning G. Active smoking and risk of metabolic syndrome: A meta-analysis of prospective studies. *PLoS One* 2012;7:e47791.
9. Faria G, Gonçalves A, Cunha R, et al. Beyond central adiposity: Liver fat and visceral area are associated with metabolic syndrome in morbidly obese patients. *Int J Surg* 2015;14:75–79.
10. Ledoux S, Coupaye M, Essig M, et al. Traditional anthropometric parameters still predict metabolic disorders in women with severe obesity. *Obesity* 2010;18:1026–1032.
11. Zazai R, Wilms B, Ernst B, et al. Waist circumference and related anthropometric indices are associated with metabolic traits in severely obese subjects. *Obes Surg* 2014; 24:777–782.
12. Ebron K, Andersen CJ, Aguilar D, et al. A larger body mass index is associated with increased atherogenic dyslipidemia, insulin resistance, and low-grade inflammation in individuals with metabolic syndrome. *Metab Syndr Relat Dis* 2015;13:458–464.
13. Kramer CK, Zinman B, Fetnakaran R. Are metabolically healthy overweight and obesity benign conditions? A systematic review and meta-analysis. *Ann Intern Med* 2013; 159:758–769.
14. Gonçalves A, Glade MJ, Meguid MM. Metabolically healthy obese individuals: Key protective factors. *Nutrition* 2016;32:14–20.
15. Robberecht H, Hermans N. Biomarkers of metabolic syndrome: Biochemical background and clinical significance. *Metab Syndr Relat Dis* 2016;14:47–93.

16. Tinahones FJ, Garrido-Sánchez L, Murri M. Particular characteristics of the metabolic syndrome in patients with morbid obesity. *Endocrinol Nutr* 2013;60:127–135.
17. Tinahones FJ, et al. Obesity and insulin resistance-related changes in the expression of lipogenic and lipolytic genes in morbidly obese subjects. *Obes Surg* 2010;20:1559–1567.
18. Sung K-C, Wild SH, Byrne CD. Lipoprotein (a), metabolic syndrome and coronary calcium score in a large occupational cohort. *Nutr Metab Cardiovasc Dis* 2013;23:1239–1246.
19. Marzano L, Colussi GL, De Torre M, et al. Relationships of plasma lipoprotein (a) levels with insulin resistance in hypertensive patients. *Metabolism* 2014;63:1439–1446.
20. Van Pelt RE, Evans EM, Schechtman KB, et al. Contributions of total and regional fat mass to risk for cardiovascular disease in older women. *Am J Physiol Endocrinol Metab* 2002;282:E1023–E1028.
21. Aasen G, Fagertun H, Tonstad S, et al. Leg fat mass as measured by dual X-ray absorptiometry (DXA) impacts insulin resistance differently in obese women versus men. *Scand J Clin Lab Invest* 2009;69:181–189.
22. Faliola E, Tirabassi G, Canibus P, et al. Protective effect of leg fat against cardiovascular risk factors in obese premenopausal women. *Nutr Metab Cardiovasc Dis* 2009;19:39–44.
23. Sung K-C, Cha S-C, Sung J-W, et al. Metabolically healthy obese subjects are at risk of fatty liver but not of preclinical atherosclerosis. *Nutr Metab Cardiovasc Dis* 2014;24:256–262.

Address correspondence to:
Sasa Dusanov, MD
Section for Preventive Cardiology
Department of Endocrinology
Morbid Obesity and Preventive Medicine
Oslo University Hospital
P.b. 4956 Nydalen
Oslo N-0424
Norway

E-mail: sasdus@ous-hf.no

Nutrition, Metabolism & Cardiovascular Diseases (2018) **28**, 735–742

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Associations between persistent organic pollutants and metabolic syndrome in morbidly obese individuals

S. Dusanov [a,*], J. Ruzzin [b], H. Kiviranta [c], T.O. Klemsdal [a], L. Retterstøl [d], P. Rantakokko [c], R. Airaksinen [c], S. Djurovic [e,f], S. Tonstad [a]

[a] Section for Preventive Cardiology, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, P.b. 4956 Nydalen, N-0424, Oslo, Norway
[b] Department of Biology, University of Bergen, Bergen, Norway
[c] National Institute for Health and Welfare, THL, Department of Health Security, P.O. Box 95, FI-70701, Kuopio, Finland
[d] Department of Medical Genetics, Oslo University Hospital, Oslo, Norway
[e] Department of Medical Genetics, Oslo University Hospital, Bergen, Norway
[f] KG Jebsen Centre for Psychosis Research, Department of Clinical Science, University of Bergen, Bergen, Norway

Received 15 January 2018; received in revised form 5 March 2018; accepted 6 March 2018
Handling editor: A. Siani
Available online 13 March 2018

KEYWORDS
Metabolic syndrome;
POPs;
Morbid obesity;
Insulin resistance

Abstract *Background and aims:* Persons with "metabolically healthy" obesity may develop cardiometabolic complications at a lower rate than equally obese persons with evident metabolic syndrome. Even morbidly obese individuals vary in risk profile. Persistent organic pollutants (POPs) are widespread environmental chemicals that impair metabolic homeostasis. We explored whether prevalence of metabolic syndrome in morbidly obese individuals is associated with serum concentrations of POPs.
Methods and results: A cross-sectional study among 161 men and 270 women with BMI >35 kg/m^2 and comorbidity, or >40 kg/m^2. Circulating concentrations of 15 POPs were stratified by number of metabolic syndrome components. In multiple logistic regression analysis odds ratios between top quartile POPs and metabolic risk factors versus POPs below the top quartile were calculated adjusting for age, gender, body mass index, smoking status, alcohol consumption and cholesterol concentrations. Age-adjusted concentrations of *trans*-nonachlor and dioxin-like and non-dioxin-like polychlorinated biphenyls (PCBs) increased with number of metabolic syndrome components in both genders ($p < 0.001$), while the organochlorine pesticides HCB, β-HCH and p,p'DDE increased only in women ($p < 0.008$). Organochlorine pesticides in the top quartile were associated with metabolic syndrome as were dioxin-like and non-dioxin-like PCBs (OR 2.3 [95% CI 1.3–4.0]; OR 2.5 [95% CI 1.3–4.8] and 2.0 [95% CI 1.1–3.8], respectively). Organochlorine pesticides were associated with HDL cholesterol and glucose (OR = 2.0 [95% CI = 1.1–3.4]; 2.4 [95% CI = 1.4–4.0], respectively). Dioxin-like PCBs were associated with diastolic blood pressure, glucose and homeostatic model assessment-insulin resistance index (OR = 2.0 [95% CI = 1.1–3.6], 2.1 [95% CI = 1.2–3.6] and 2.1 [95% CI = 1.0–4.3], respectively).
Conclusion: In subjects with morbid obesity, metabolic syndrome was related to circulating levels of organochlorine pesticides and PCBs suggesting that these compounds aggravate clinically relevant complications of obesity.

* Corresponding author.
E-mail address: sasdus@ous-hf.no (S. Dusanov).

https://doi.org/10.1016/j.numecd.2018.03.004
0939-4753/

Introduction

Obesity represents one of the most widespread public health and clinical challenges worldwide [1], and is commonly associated with a cluster of cardiometabolic risk factors [2]. Notably, obesity is not always associated with metabolic syndrome, even among persons characterized as morbidly obese (BMI of $\geq$40 kg/m^2 or $\geq$ 35 and with obesity related health conditions). Obese persons that do not meet criteria for metabolic syndrome have been characterized as "metabolically healthy obese" [3]. While these individuals are at risk for type 2 diabetes and cardiovascular events, they may be at lower risk than persons with metabolic syndrome, at the same level of obesity, at least in the short term [4–6]. Why some obese persons develop cardiometabolic risks earlier or more markedly than others may be related to lifestyle or polygenic variability but remains poorly understood.

During the last years, emerging evidence has linked environmental pollutants, particularly persistent organic pollutants (POPs), to obesity and related health problems [7]. Among POPs, organochlorine pesticides and polychlorinated biphenyls (PCBs) are lipophilic chemicals that accumulate in living organisms, acting as endocrine and metabolic disruptors [8]. Though the manufacture and usage of many of these compounds have been regulated, the prior environmental burden may influence current body levels and result in hazardous health conditions.

Results of observational studies have shown that serum concentrations of POPs are related to risk of type 2 diabetes [7,8]. Furthermore, in persons without diabetes, organochlorine pesticides and PCBs have been associated with insulin resistance, dyslipidemia and metabolic syndrome in the United States [9–12]. These relationships have been confirmed in studies that analyzed adipose depots of POPs [13,14]. Even in normal weight individuals POP concentrations appear to associate with unhealthy metabolic phenotypes [15]. Interestingly, the burden of POPs may be more strongly related to some metabolic risks than others [11–16] and may correlate with degree of atherosclerosis [17]. Accumulated POPs may differ according to population characteristics, including ethnic and socioeconomic variables [18].

In persons with already established obesity, some data have shown associations between POPs and metabolic risks. In obese postmenopausal women the metabolically healthy obese phenotype was associated with lower concentrations of POPs compared to metabolically abnormal phenotypes [19]. In patients that underwent bariatric surgery, POPs concentrations in adipose tissue were linked to metabolic syndrome [13].

Patients with morbid obesity bear the largest burden of excess body fat. However, even in this high-risk group, metabolic risks may vary therefore requiring a range of clinical approaches to treatment [20]. The current study examined whether circulating concentrations of POPs were associated with metabolic syndrome in a sample of consecutive men and women with morbid obesity referred for treatment.

Methods

Patients referred to the Preventive Cardiology Clinic at Oslo University Hospital, Oslo, Norway between April 2005 and December 2010 were asked to participate, and included provided that they gave written informed consent. The study conformed to the Helsinki Declaration and was evaluated by the Ethics committee for region 1 in Norway.

Participants with BMI $\geq$35 kg/m^2 with accompanying obesity-related comorbidity (including hypertension, sleep apnea, dyspnea, polycystic ovarian syndrome, asthma, hypercholesterolemia, gout, musculoskeletal symptoms, gall bladder symptoms, esophageal reflux, pulmonary or deep vein embolism, intermittent claudication, angina pectoris and depression) or BMI $\geq$40 kg/m^2 regardless of comorbidity were included in the present study (n = 431). Subjects with type 2 diabetes were excluded as the focus of the present study was on cardiometabolic risks.

Participants completed a health questionnaire and underwent anthropometric measurements. Waist circumference was measured at midpoint between the inferior costal margin and the highest point of the iliac crest, and hip circumference was measured at the widest point around the hips. Height was recorded to the nearest cm. Patients were weighed to the nearest 1.0 kg using a calibrated mobile electronic scale (Seca 720, Medical Scales and Measuring Systems, Birmingham, UK) and BMI was calculated. Blood pressure was measured using an automatic blood pressure monitor (52000 Series Vital Signs Monitor, Welch Ally, New York, USA) following a 5-min rest.

Classification of metabolic syndrome was performed according to the harmonized definition [2] and included $\geq$3 of the following: waist circumference $\geq$102 cm for men and $\geq$88 cm for women; blood pressure $\geq$130/85 mmHg or drug-treated hypertension; triglycerides $\geq$1.7 mmol/l; HDL-cholesterol $\leq$1.0 mmol/l for men or $\leq$1.3 mmol/l for women; and fasting glucose $\geq$5.6 mmol/l. Subjects were stratified by number of these components.

Laboratory analyses

Participants fasted overnight for $\geq$10 h, before providing blood samples between 0800 h and 1100 h. Analyses of blood samples were performed at Oslo University Hospital (Clinical Chemistry Laboratory at Ullevål and Endocrine Laboratory at Aker). Total cholesterol, HDL-cholesterol, triglycerides, glucose, and high-sensitivity C-reactive protein (CRP) concentrations were measured on an automated analyzer Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). Insulin was determined by non-competitive immunofluorometric assay, using an AutoDelfia 1235 Automatic Immunoassay System (H1855-21291) (Perkin Elmer Inc.) in 209 subjects with BMI $\geq$40 kg/m^2. The Homeostasis Model Assessment insulin resistance index (HOMA-IR) to estimate insulin resistance was calculated in subjects with insulin measurements [21].

Assessment of circulating POPs concentrations

POPs were measured in 200 μl of serum at the National Institute for Health and Welfare (THL), Kuopio, Finland. The following POPs were measured: polybrominated diphenyl ethers (PBDEs) 47, 99 and 153; polychlorinated biphenyls (PCBs) including dioxin-like (118, 156) and non-dioxin-like (74, 99, 138, 153, 170, 180, 183, 187) compounds; and organochlorine pesticides including dichlorodiphenyltrichloroethane (p,p'DDT), dichlorodiphenyldichloroethylene (p,p'DDE), hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH), pentachlorobenzene (PeCB), hexachlorobenzene, *trans*-nonachlor, and oxychlordane. The detection rate for PeCB, α-HCH, γ-HCH, oxychlordane and all PBDEs was <75%, and thus, these analytes were excluded from further statistical analyses.

A full description of the analytical method has been published previously [22]. Limits of quantification for POPs were 5–40 pg/ml. In each batch of samples a control serum sample from the National Institute of Standard and Technology, Standard Reference Material (SRM) 1958 for POPs was included (n = 13). An in-house produced low level control sample was prepared by 1–9 dilution of SRM 1958 with new born calf serum (NBCS) and also included in each batch of samples (n = 13). Average concentrations for POPs from SRM 1958 were 87–111% of the certified/indicative concentrations. Coefficients of variation (CVs) were 1.4–4.4% for SRM 1958 and 1.8–8.9% for diluted SRM 1958. The Environmental Health Unit participates to AMAP inter laboratory comparison (Ring Test for Persistent Organic Pollutants in human serum, National Institute of Public Health, Quebec, Canada) where 16 of the target POPs from three serum samples are reported for each round. During the last two years results from all samples for all POPs have been acceptable (|z|<2). Accuracy of the results for individual compounds from individual samples varied from 77 to 121% of the assigned values.

Statistical analyses

Statistical analyses were performed using SPSS 21 (SPSS Inc, Chicago, IL). P-values <0.05 were considered statistically significant. All variables were tested for normality, and logarithmically transformed if not normally distributed (triglycerides, BMI, HOMA-IR index, CRP and all POPs). Elevated CRP concentrations were defined as ≥4.0 mg/L versus <4.0 mg/L. CRP concentrations ≥40 mg/l were excluded due to likely intercurrent illness (n = 7).

We calculated sums of five organochlorine pesticides (HCB, β-HCH, *trans*-nonachlor, p,p'DDT and p,p'DDE), sums of dioxin-like PCBs (118 and 156) and non-dioxin-like PCBs (74, 99, 138, 153, 170, 180, 183 and 187). Age-adjusted partial correlations between sums of POPs and BMI and total cholesterol were calculated as were bivariate correlations with age. Age-adjusted means were compared for women and men according to number of metabolic syndrome components using one-way analysis of variance (ANOVA). The 75th percentile of each organochlorine pesticide was calculated in each sex. A point was given for each organocholorine pesticide ≥75th percentile, the sum of points was calculated and the 75th percentile of the sum calculated. Dioxin-like PCBs, and non-dioxin-like PCBs were summed and the sex-specific 75th percentile was calculated.

We examined the mean of each metabolic syndrome component and its markers according to tertiles of each organochlorine pesticide, sums of organochlorine pesticides, and sums of dioxin-like and non-dioxin-like PCBs. Values of metabolic syndrome components or markers in the lowest tertiles similar to the highest tertiles were not observed, and further analyses of possible U-shaped relationships were not done.

Finally we performed binary logistic regression analysis controlling for age, gender, smoking status, body mass index, alcohol consumption and cholesterol concentrations to estimate odds ratios and 95% confidence intervals for presence of metabolic syndrome, its components and markers according to the presence of elevated POPs (separately for organochlorine pesticides and summed for organochlorine pesticides and dioxin-like and non-dioxin-like PCBs). POP concentrations adjusted for total cholesterol were compared to those obtained when cholesterol was included as a covariate in the models without cholesterol-adjusted concentrations. These results did not differ substantially (data not shown) and results considering total cholesterol a covariate in the regression analyses are presented.

Results

We analyzed data from 431 participants (270 women and 161 men) aged between 18 and 78 years. Clinical characteristics and cardiometabolic risk markers are presented in Table 1. The mean BMI was 42 kg/m^2, and almost three-fourths of men and 60% of women met metabolic syndrome criteria.

In women, concentrations of all POPs, with the exception of p,p'DDT, increased with number of metabolic syndrome components (Table 2). In men, dioxin-like and non-dioxin-like PCB concentrations increased according to number of metabolic syndrome components, whereas, among organochlorine pesticides, only *trans*-nonachlor concentrations were statistically significantly increased (Table 3).

Sums of organochlorine pesticides, dioxin-like PCBs and non-dioxin-like PCBs correlated strongly with age (r = 0.5, 0.5, 0.6, respectively; p < 0.0001). However, summed organochlorine pesticides, dioxin-like PCBs and non-dioxin-like PCBs did not correlate with BMI in partial correlation analyses corrected for age among men (r = −0.01, p = 0.9; r = 0.05, p = 0.6 and r = 0.01, p = 0.9; respectively) and women (r = 0.01, p = 0.9; r = −0.01, p = 0.8 and r = −0.1, p = 0.1; respectively) or with waist circumference in men (r = −0.1, p = 0.1; r = −0.03, p = 0.6 and r = −0.1, p = 0.4; respectively) and women (r = −0.1, p = 0.3; r = 0.1, p = 0.1 and r = 0.1, p = 0.1; respectively). Summed organochlorine pesticides did not correlate with total cholesterol (r = 0.1, p = 0.2), while

Table 1 Characteristics of participants (N = 431) stratified by gender. Values are shown as mean (SD) except for triglycerides, C-reactive protein (CRP), alanine aminotransferase (ALT) and homeostatic model assessment-insulin resistance (HOMA-IR) index which are shown as median and 25. and 75. percentiles. Smoking status, metabolic syndrome and use of antihypertensive drugs are shown in percentages.

	Men (n = 161)	Women (n = 270)
Age, years	41.5 (12.3)	39.7 (11.9)
BMI, kg/m^2	41.6 (5.1)	42.4 (5.1)
Smoking, No (percentage)	47 (29.2)	29 (21.9)
Metabolic syndrome, No (percentage)	116 (72.0)	160 (59.3)
Use of statin, No (percentage)	24 (14.9)	16 (5.9)
Components of metabolic syndrome		
Waist circumference, cm	132 (13)	119 (13)
Systolic blood pressure, mmHg	137 (16)	130 (16)
Diastolic blood pressure, mmHg	87 (10)	84 (10)
Use of antihypertensive drugs, No (percentage)	57 (35.4)	62 (23.0)
HDL-cholesterol, mmol/l	1.1 (0.3)	1.3 (0.3)
Triglycerides, mmol/l	1.8 (1.3–2.6)	1.4 (1.0–1.9)
Fasting glucose, mmol/l	5.5 (0.6)	5.3 (0.6)
Other cardiometabolic markers		
Total cholesterol, mmol/l	5.1 (1.0)	5.3 (1.1)
HOMA-IR index[a]	4.8 (3.2–7.0)	3.2 (2.1–4.8)
CRP, mg/L[b]	5.0 (2.0–8.0)	7.0 (4.0–12.0)
ALT, U/L	45.0 (34.0–63.0)	24.0 (19.0–34.0)

[a] For HOMA-IR index n = 76 for men and n = 133 for women.
[b] For CRP 5 values were missing and 7 values > 40 mg/L were excluded.

summed dioxin-like PCBs and non-dioxin-like PCBs correlated with total cholesterol in partial correlations corrected for age (both r = 0.2, p < 0.0001).

In regard to single pesticides showing significant associations in Table 2 or 3, HCB and β-HCH concentrations ≥75th percentile were associated with fasting glucose (OR = 2.0 [CI = 1.2–3.4] and 2.0 [CI = 1.2–3.3], respectively. *Trans*-nonachlor was associated with metabolic syndrome (OR 2.1 [1.2–3.9] and triglycerides (OR 1.9 [1.1–3.5]. p,p'DDE showed no significant associations (data not shown).

Organochlorine pesticides ≥75th percentile were associated with metabolic syndrome, HDL cholesterol and glucose (Table 4). As shown in Table 5, dioxin-like PCBs ≥75th percentile were associated with metabolic syndrome, diastolic blood pressure and fasting glucose, as well as with HOMA-IR index. Non-dioxin-like PCBs were associated with metabolic syndrome and fasting glucose (but pre-specified statistical significance was not reached for glucose) as shown in Table 6. Organochlorine pesticides ≥75th percentile were not associated with elevated CRP concentrations in men and women (OR = 0.8 [CI = 0.3–2.1] and OR = 0.8 [CI = 0.4–1.6], respectively). Likewise, dioxin-like PCBs were not associated with CRP in men and women (OR = 0.5 [CI = 0.2–1.6] and OR = 0.8 [CI = 0.4–1.8], respectively). Non-dioxin-like PCBs were associated with lower risk of high CRP in men (OR = 0.3 [CI = 0.09–0.7]), but not in women (OR = 0.9 [CI = 0.4–2.1]).

Discussion

In this study conducted in morbidly obese men and women, the odds of metabolic syndrome (i.e. fulfilling three or more of the criteria) was increased by 2.3 times in participants with high circulating concentrations of organochlorine pesticides, 2.5 times in participants with

Table 2 Persistent organic pollutant concentrations in women according to number of metabolic syndrome components (n = 270). Age-adjusted means, median and 25. and 75. percentiles are shown.

	1-2 (n = 110) Mean 50. (25., 75.)		3 (n = 83) Mean 50. (25., 75.)		4 (n = 59) Mean 50. (25., 75.)		5 (n = 18) Mean 50. (25., 75.)		P value
Organochlorine pesticides (pg/ml)									
HCB	75.3	54.5 (37.0–79.3)	66.8	75.0 (46.0–112.0)	78.5	76.0 (53.0–101.0)	84.4	83.5 (59.3–123.3)	0.001
β-HCH	22.9	22.0 (8.0–45.3)	32.7	35.0 (22.0–63.0)	30.4	31.0 (19.0–53.0)	47.6	51.5 (27.5–72.3)	0.003
trans-Nonachlor	11.9	11.0 (6.0–21.0)	16.5	19.0 (11.0–38.0)	19.5	18.0 (10.0–37.0)	25.3	32.5 (15.0–54.3)	<0.0001
p,p'DDT	11.3	8.0 (8.0–8.0)	13.6	8.0 (8.0–22.0)	13.8	8.0 (8.0–20.0)	19.6	20.0 (8.0–29.3)	0.1
p,p'DDE	315	265 (151–597)	407	466 (227–1044)	417	335 (175–722)	679	715 (327–1269)	0.008
Dioxin-like polychlorinated biphenyls (pg/ml)									
PCB-118	26.8	27.0 (15.0–41.8)	34.1	38.0 (21.0–67.0)	43.0	40.0 (26.0–71.0)	44.3	51.0 (28.8–83.3)	<0.0001
PCB-156	9.7	9.5 (3.0–20.0)	13.6	18.0 (8.0–33.0)	15.1	16.0 (8.0–26.0)	17.2	18.5 (9.0–34.0)	<0.0001
∑	37.5	39.0 (19.8–59.3)	55.1	61.0 (29.0–95.0)	57.7	59.0 (38.0–97.0)	63.7	74.0 (41.8–116.3)	<0.0001
Nondioxin-like polychlorinated biphenyls (pg/ml)									
PCB-74	8.9	9.5 (3.0–15.0)	12.0	16.0 (7.0–27.0)	14.5	14.0 (8.0–25.0)	15.4	19.0 (10.0–23.3)	<0.0001
PCB-99	11.3	12.0 (6.8–19.0)	14.5	17.0 (9.0–33.0)	17.4	19.0 (10.0–30.0)	18.8	18.0 (11.8–40.3)	0.005
PCB-138	70.4	76.5 (37.0–121.0)	90.6	113.0 (54.0–187.0)	106.8	114.0 (63.0–164.0)	112.6	116.0 (71.5–226.3)	0.002
PCB-153	104	106 (53–182)	139	180 (82–291)	159	164 (93–247)	173	183 (109–334)	<0.0001
PCB-170	28.6	29.5 (14.8–56.3)	38.1	51.0 (23.0–87.0)	44.9	46.0 (24.0–76.0)	49.5	55.0 (31.5–92.3)	<0.0001
PCB-180	60.2	62.0 (29.0–117.0)	81.5	103.0 (49.0–185.0)	92.9	98.0 (52.0–153.0)	104.3	116.5 (67.3–196.8)	<0.0001
PCB-183	7.1	7.5 (3.0–12.3)	9.4	12.0 (5.0–21.0)	11.2	11.0 (6.0–19.0)	13.0	12.0 (8.0–28.3)	<0.0001
PCB-187	17.9	19.0 (9.0–34.5)	23.9	31.0 (14.0–59.0)	29.4	32.0 (16.0–52.0)	32.7	35.5 (21.8–70.3)	<0.0001
∑	312	316 (157–566)	469	544 (252–875)	468	491 (274–768)	535	549 (323–1020)	<0.0001

HCB = Hexachlorobenzene, HCH-beta = β-hexachlorocyclohexane, p,p'DDT = dichlorodiphenyltrichloroethane, p,p'DDE = Dichlorodiphenyldichloroethylene, PCB = polychlorinated biphenyl.

Table 3 Persistent organic pollutant concentrations in men according to number of metabolic syndrome components (N = 161). Age-adjusted means, median and 25. and 75. percentiles are shown.

	1-2 (n = 45) Mean 50 (25., 75.)		3 (n = 45) Mean 50 (25., 75.)		4 (n = 45) Mean 50 (25., 75.)		5 (n = 26) Mean 50 (25., 75.)		P-value
Organochlorine pesticides (pg/ml)									
HCB	73.2	67.2 (42.5–94.0)	78.0	77.0 (59.5–99.5)	84.8	86.0 (59.0–125.0)	101.2	94.0 (83.8–142.0)	0.08
β-HCH	27.2	23.0 (8.0–41.0)	27.3	27.0 (17.0–43.5)	30.5	36.0 (17.5–57.5)	37.3	42.0 (32.5–75.5)	0.4
trans-Nonachlor	19.2	18.0 (8.0–32.0)	30.1	30.0 (14.0–52.5)	31.1	34.0 813.5–72.0)	35.9	46.5 (32.8–69.3)	0.001
p,p'DDT	11.7	8.0 (8.0–17.5)	11.6	8.0 (8.0–16.5)	11.6	8.0 (8.0–18.5)	15.1	16.0 (8.0–26.8)	0.3
p,p'DDE	358	321 (131–651)	398	358 (204–707)	454	360 (195–902)	598	690 (431–1297)	0.1
Dioxin-like polychlorinated biphenyls (pg/ml)									
PCB-118	36.3	30.0 (17.5–59.0)	48.7	44.0 (31.5–80.0)	44.2	44.0 (23.5–80.0)	58.9	77.0 (49.3–119.3)	0.007
PCB-156	16.8	14.0 (7.0–32.5)	23.8	23.0 (14.5–45.0)	24.8	32.0 (10.5–57.0)	32.7	46.5 (27.8–69.0)	<0.0001
∑	45.8	50.0 (24.5–87.5)	70.8	73.0 (50.0–125.0)	75.9	76.0 (35.0–151.0)	121.5	124.5 (83.8–204.8)	0.001
Non-dioxin-like polychlorinated biphenyls (pg/ml)									
PCB-74	11.8	10.0 (5.0–21.0)	15.7	15.0 (9.0–25.0)	15.0	17.0 (8.0–31.0)	22.4	25.0 (16.5–43.8)	0.007
PCB-99	16.2	16.0 (8.0–26.0)	23.3	25.0 (13.5–40.0)	23.0	25.0 (14.5–46.0)	33.2	44.5 (27.0–55.8)	0.002
PCB-138	109	96 (49–181)	150	142 (92–252)	152	167 (80–322)	206	266 (177–362)	<0.0001
PCB-153	167	150 (75–293)	232	204 (125–366)	238	276 (120–469)	317	394 (291–583)	<0.0001
PCB-170	47.6	42.0 (21.5–86.0)	68.1	68.0 (35.5–121.5)	71.4	95.0 (35.5–146.0)	94.8	124.0 (82.5–177.0)	<0.0001
PCB-180	102	88 (47–184)	145	141 (77–265)	150	196 (73–312)	197	262 (176–388)	<0.0001
PCB-183	11.3	9.0 (3.0–19.0)	16.2	15.0 (9.0–29.5)	15.3	18.0 (8.0–37.5)	23.0	28.5 (20.0–40.3)	0.001
PCB-187	29.2	24.0 (12.5–55.5)	43.9	43.0 (24.5–85.0)	45.3	58.0 (20.5–102.5)	61.9	85.0 (49.5–126.3)	<0.0001
∑	416	445 (227–910)	671	660 (417–1196)	771	878 (362–1440)	1287	1233 (655–1796)	<0.0001

HCB = Hexachlorobenzene, HCH-beta = β-hexachlorocyclohexane, p,p'DDT = dichlorodiphenyltrichloroethane, p,p'DDE = Dichlorodiphenyldichloroethylene, PCB = polychlorinated biphenyl.

high circulating concentrations of dioxin-like PCBs and doubled in those with elevated non-dioxin-like PCB concentrations compared to those with lower circulating concentrations. In participants with elevated organochlorine compounds HDL-cholesterol was decreased and fasting glucose concentrations were elevated. In participants with elevated dioxin-like PCBs, diastolic blood pressure and fasting glucose concentrations were elevated, as was HOMA-IR, indicating insulin resistance. To our knowledge, this is the first study to explore the relation between POPs concentrations and metabolic risks in the morbidly obese.

Table 4 Odd ratios of ranked organochlorine pesticides (HCB, β-HCH, *trans*-Nonachlor, p,p'DDT, p,p'DDE) equal or higher than the gender-specific 75th percentile for presence of metabolic syndrome and metabolic risk factors adjusted for age, gender, BMI, smoking, alcohol consumption and total cholesterol concentration (N = 431).

	Odds ratio	95% Confidence interval	P value
Metabolic syndrome (≥3 components vs <3)	2.3	1.3–4.0	0.006
Components of metabolic syndrome			
Systolic BP, (≥130 mm Hg or use of medication vs <130 mmHg)	0.6	0.3–1.0	0.1
Diastolic BP, (≥85 mm Hg or use of medication vs <85 mmHg)	0.9	0.5–1.5	0.6
HDL-cholesterol (low versus high)[a]	2.0	1.1–3.4	0.02
Triglycerides (≥1.7 mmol/l vs <1.7 mmol/l)	1.6	0.9–2.8	0.1
Fasting glucose (≥5.6 mmol/l vs <5.6 mmol/l)	2.4	1.4–4.0	0.001
Other cardiometabolic markers			
HOMA-IR index[b] (≥median vs <median)	1.9	1.0–3.7	0.1
ALT (high versus low)[c]	0.7	0.3–1.4	0.3

[a] For men <1.0 mmol/l vs ≥1.0 mmol/l. For women <1.3 mmol/l vs ≥1.3 mmol/l.
[b] For HOMA-IR index (homeostatic model assessment-insulin resistance) n = 209.
[c] For men >70 U/L vs ≤70 U/L. For women >45 U/L vs ≤45 U/L.

The population studied was patients consecutively referred for treatment of morbid obesity, and thus may represent a higher cardiovascular risk population than the general obese population. Despite likely selection bias, over one-quarter of men and 40% of women did not meet the definition of metabolic syndrome, as they demonstrated only 1–2 components, including waist circumference, in all cases, and none or only one more metabolic syndrome component. These differing profiles illustrate the importance of understanding possible underlying pathophysiological mechanisms that may expose some individuals to greater risk of metabolic disturbances and in turn elevated cardiovascular risk.

We found that age-adjusted concentrations of POPs increased in a near-linear manner with number of metabolic syndrome components (Tables 2 and 3). Concentrations of POPs were strongly age-related reflecting the effects of biological aging or birth cohort effects. Lipophilic compounds accumulate in fat deposits with time and older patients were exposed to high exposures at a time when environmental levels were higher than today. Health effects are influenced by concentrations experienced by human cohorts during several decades [20]. Furthermore, the lifetime accumulation of POPs in serum or adipose tissue reflects common dietary sources over a long period of time.

A number of previous studies in non-diabetic subjects have found associations between POPs and dyslipidemia and metabolic syndrome or high fasting glucose

Table 5 Odd ratios of metabolic syndrome and metabolic risk factors according to summed dioxin-like PCBs equal or higher than the gender-specific 75th percentile adjusted for age, gender, BMI, smoking, alcohol consumption and total cholesterol concentration (N = 431).

	Odds ratio	95% Confidence interval	P value
Metabolic syndrome (≥3 components vs <3)	2.5	1.3–4.8	0.005
Components of metabolic syndrome			
Systolic BP, (≥130 mm Hg or use of medication vs <130 mmHg)	1.5	0.8–2.8	0.2
Diastolic BP, (≥85 mm Hg or use of medication vs <85 mmHg)	2.0	1.1–3.6	0.02
HDL-cholesterol (low versus high)[a]	1.4	0.8–2.4	0.3
Triglycerides (≥1.7 mmol/l vs <1.7 mmol/l)	1.7	0.9–3.1	0.1
Fasting glucose (≥5.6 mmol/l vs <5.6 mmol/l)	2.1	1.2–3.6	0.01
Other cardiometabolic markers			
HOMA-IR index[b] (≥median vs <median)	2.1	1.0–4.3	0.047
ALT (high versus low)[c]	0.9	0.4–2.0	0.8

[a] For men <1.0 mmol/l vs ≥ 1.0 mmol/l. For women <1.3 mmol/l vs ≥1.3 mmol/l.
[b] For HOMA-IR index (homeostatic model assessment-insulin resistance) n = 209.
[c] For men >70 U/L vs ≤70 U/L. For women >45 U/L vs ≤45 U/L.

concentrations [10,12,23]. However, the specific POPs or group of POPs associated with cardiometabolic risk may vary according to geographic location and time period. We grouped POPs into three categories (organochlorine pesticides, dioxin-like PCBs and non-dioxin-like PCBs) ≥ the 75th percentile threshold as looking at each compound separately may result in misclassification [24,25] and multiple hypothesis testing, with the exception of organochlorine pesticides, that we also looked at separately. PCBs and hexachlorobenzene concentrations predicted type 2 diabetes in meta-analysis [26] in line with our findings. PCBs have been associated with impaired glucose metabolism in young adults followed over 23 years [27] and cross-sectionally in obese individuals [28]. Recently, a mixture of PCBs and hexachlorobenzene was shown to have an additive effect on incident metabolic syndrome, but synergistic effects were not found [23]. A study of POPs in adipose tissue samples obtained between 2003 and 2004 found independent associations between the organochlorine pesticides β-hexachlorocyclohexane and hexachlorobenzene and metabolic disturbances [12], both compounds that associated with impaired fasting glucose in the present study. In the National Health and Nutrition Examination Survey performed in 1999–2002 organochlorine pesticides were most strongly associated with HOMA-IR and metabolic syndrome [9,10], but associations were also found for some PCBs [9,10]. The association with organochlorine pesticides was strengthened as waist circumference increased [9,10]; in contrast we did not find a significant association between waist circumference and organochlorine pesticides in morbidly obese individuals.

Table 6 Odd ratios of metabolic syndrome and metabolic risk factors according to summed non-dioxin-like PCBs equal or higher than the gender-specific 75th percentile adjusted for age, gender, BMI, smoking, alcohol consumption and total cholesterol concentration (N = 431).

	Odds ratio	95% Confidence interval	P value
Metabolic syndrome (≥3 components vs <3)	2.0	1.1–3.8	0.03
Components of metabolic syndrome			
Systolic BP, (≥130 mm Hg or use of medication vs <130 mmHg)	1.1	0.6–2.1	0.7
Diastolic BP, (≥85 mm Hg or use of medication vs <85 mmHg)	1.6	0.9–2.8	0.1
HDL-cholesterol (low versus high)[a]	1.2	0.7–2.2	0.5
Triglycerides (≥1.7 mmol/l vs <1.7 mmol/l)	1.7	0.9–3.1	0.1
Fasting glucose (≥5.6 mmol/l vs <5.6 mmol/l)	1.8	1.0–3.1	0.05
Other cardiometabolic markers			
HOMA-IR index[b] (≥median vs <median)	1.7	0.8–3.4	0.2
ALT (high versus low)[c]	0.9	0.4–2.0	0.9

[a] For men <1.0 mmol/l vs ≥1.0 mmol/l. For women <1.3 mmol/l vs ≥1.3 mmol/l.
[b] For HOMA-IR index (homeostatic model assessment-insulin resistance) n = 209.
[c] For men >70 U/L vs ≤70 U/L. For women >45 U/L vs ≤45 U/L.

Some associations of POPs and other components of metabolic syndrome were observed. We found as association between elevated organochlorine pesticides and low HDL-cholesterol. This relation was also observed in a prospective study of ten top ranked POPs, including hezachlorobenzene [23]. Furthermore, we found an association between dioxin-like PCBs and diastolic blood pressure. Organochlorine pesticides have been shown to increase risk of hypertension in overweight Spanish subjects over a 10-year follow-up period [29]. A study showed that POPs concentrations in the second and third tertile were associated with systolic and diastolic blood pressure in linear regression tests in persons with hypertension, when adjusted for covariates, while in normotensive persons' blood pressure was related to the top tertile POPs concentration [30]. Persistent PCBs with ≥7 chlorines may be more predictive than other PCBs [12] but we did not find this to be the case in the current population (data not shown).

Inverted U-shaped associations between POPs and metabolic syndrome have been reported in a number of studies [10–12]. In obese patients, a previous study found a U-shaped association between p,p'DDE concentrations and type 2 diabetes [31]. We did not find U-shaped associations in the current study. Surprisingly, both dioxin-like and non-dioxin-like PCBs were inversely (though very weakly and not statistically significantly) associated with BMI. A dilution effect of very high BMIs on POPs has been described. A simple model has been suggested that predicts a *negative* correlation between organochlorine pesticides levels and BMI during a period when exposures

are ongoing, as long as absorption (a) exceeds elimination rate and (b) is comparable across the population [32].

The finding of inverse relation between concentrations of non-dioxin-like PCBs and CRP concentrations in men was unexpected. However, in the present study CRP concentrations tended to be lower as the number of metabolic syndrome factors increased (data not shown). Similar inverse relationships were seen in a cross-sectional study of non-diabetic participants in the United States survey [33]. In this study, only organochlorine pesticides were positively associated with CRP concentrations. Furthermore CRP was associated with HOMA-IR only in subjects with high POPs, possibly indicating effect modification by POPs. In a study of morbidly obese persons who underwent liver biopsy during bariatric surgery, concentrations of PCB-118 and other POPs were inversely associated with lobular inflammation prior to surgery as were alanine aminotransferase concentrations [34]. These findings together suggest specific accumulation of POPs to the liver or adipose tissue with disease progression and suggest perhaps similar changes in the morbidly obese.

This study has some limitations. First, we did not have data regarding dietary habits or physical activity that could affect POPs concentrations. We did not normalize POPs concentrations for lipid values, though we controlled for cholesterol concentrations in regression analyses. Such normalization is controversial [35] and did not change our main results. Most importantly in a cross-sectional study we cannot establish causality for the main findings. Further, we only examined circulating levels of POPs, which possibly differ from levels in adipose tissue. In studies of adipose tissue obtained from patients undergoing obesity surgery [13] or other abdominal surgery [36], visceral adipose tissue had a higher storage capacity than subcutaneous adipose tissue indicating a possible local metabolic effect of increased lipolysis and release of free fatty acids [13,36]. Finally, given the high proportion of metabolic syndrome odds ratios used in the current study may overestimate risks.

Conclusion

In morbidly obese persons the odds of metabolic syndrome were increased with higher concentrations of dioxin-like and non-dioxin-like PCBs. Further study would be required to understand whether these relations are primarily driven through the association of POPs with HDL cholesterol, diastolic blood pressure and fasting glucose levels.

Acknowledgements

We thank Lien My Diep for statistical support, Elin Inderhaug for technical help in the biobank, Tuula Rissanen and Arja Moilanen for the laboratory analyses of POPs in THL and Teresia Wangensteen for matching the study population and biobank samples.

References

[1] Abarca-Gomez L, Abdeen ZA, Hamid ZA, Abu-Rmeileh NM, Acosta-Cazares B, Acuin C, et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet 2017; 390(10113):2627–42.

[2] Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International diabetes Federation task force on epidemiology and prevention; National heart, lung, and blood Institute; American heart Association; World heart Federation; International atherosclerosis Society; and International Association for the study of obesity. Circulation 2009;120:1640–5.

[3] Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, et al. The obese without cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). Arch Intern Med 2008;168: 1617–24.

[4] Hinnouho GM, Czernichow S, Dugravot A, Nabi H, Brunner EJ, Kivimaki M, et al. Metabolically healthy obesity and the risk of cardiovascular disease and type 2 diabetes: the Whitehall II cohort study. Eur Heart J 2015;36:551–9.

[5] Eckel N, Meidtner K, Kalle-Uhlmann T, Stefan N, Schulze MB. Metabolically healthy obesity and cardiovascular events: a systematic review and meta-analysis. Eur J Prev Cardiol 2016;23: 956–66.

[6] Lassale C, Tzoulaki I, Moons KGM, Sweeting M, Boer J, Johnson L, et al. Separate and combined associations of obesity and metabolic healthy with coronary heart disease: a pan-European case-cohort analysis. Eur Heart J 2018 Feb 1;39(5):397–406.

[7] Vassilopoulou L, Psycharakis C, Petrakis D, Tsiaoussis J, Tsatsakis AM. Obesity, persistent organic pollutants and related health problems. Adv Exp Med Biol 2017;960:81–110.

[8] Wu H, Bertrand KA, Choi AL, Hu FB, Laden F, Grandjean P, et al. Persistent organic pollutants and type 2 diabetes: a prospective analysis in the Nurses' Health Study and Meta-analysis. Environ Health Perspect 2013;121:153–61.

[9] Lee D-H, Lee I-K, Jin S-H, Steffes M, Jacobs Jr DR. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults. Diabetes Care 2007; 30:622–8.

[10] Lee D-H, Lee I-K, Porta M, Steffes M, Jacobs Jr DR. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetologia 2007;50:1841–51.

[11] Lee Y-M, Kim K-S, Kim S-A, Hong N-S, Lee S-J, Lee D-H. Prospective associations between persistent organic pollutants and metabolic syndrome: a nested case-control study. Sci Total Environ 2014; 496:219–25.

[12] Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs Jr DR. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. PLoS One 2011;6. e15977.

[13] Pestana D, Faria G, Sá C, Fernandes VC, Teixeira D, Norberto S, et al. Persistent organic pollutant levels in human visceral and subcutaneous adipose tissue in obese individuals – depot differences and dysmetabolism implications. Environ Res 2014;133:170–7.

[14] Mustieles V, Fernández MF, Martin-Olmedo P, González-Alzaga B, Fontalba-Navas A, Hauser R, et al. Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: combining a cross-sectional with a 10-year longitudinal study using a multi-pollutant approach. Environ Int 2017;104:48–57.

[15] Gasull M, Castell C, Pallarès N, Miret C, Pumarega J, Téllez-Plaza M, et al. Blood concentrations of persistent organic pollutants and unhealthy metabolic phenotypes in normal-weight, overweight, and obese individuals. Am J Epidemiol 2017 Jun 30. https://doi.org/10.1093/aje/kwx267. Epub ahead of print.

[16] Henriquez-Hernández LA, Luzardo OP, Zumbado M, Camacho M, Serra-Majem L, Álvarez-León EE, et al. Blood pressure in relation to contamination by polychlorobiphenyls and organochlorine pesticides: results from a population-based study in the Canary Islands (Spain). Environ Res 2014;135:48–54.

[17] Mangum LC, Mangum LH, Chambers JE, Ross MK, Meek EC, Wills RW, et al. The association of serum *trans*-nonachlor levels with atherosclerosis. J Toxicol Environ Health 2016;790:210–20.
[18] Pumarega J, Gasuli M, Lee D-H, Lopez T, Porta M. Number of persistent organic pollutants detected at high concentrations in blood samples of the United States population. PLoS One 2016; 11(8). e0160432.
[19] Gauthier M-S, Rabasa-Lhoret R, Prud'homme D, Karelis AD, Geng D, van Bavel B, et al. The metabolically healthy but obese phenotype is associated with lower plasma levels of persistent organic pollutants as compared to the metabolically abnormal obese phenotype. J Clin Endocrinol Metab 2014;99:E1061–6.
[20] Hopkins JL, Hopkins PN, Brinton EA, Adams TD, Davidson LE, Nanjee MN, et al. Expression of metabolic syndrome in women with severe obesity. Metab Syndr Relat Disord 2017;15:1–8.
[21] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487–95.
[22] Koponen J, Rantakokko P, Airaksinen R, Kiviranta H. Determination of selected perfluorinated alkyl acids and persistent organic pollutants from a small volume human serum sample relevant for epidemiological studies. J Chromatogr A 2013;1309:48–55.
[23] Lind L, Salihovic S, Lampa E, Lind PM. Mixture effects of 30 environmental contaminants on incident metabolic syndrome-A prospective study. Environ Int 2017;107:8–15.
[24] Porta M, Pumarega J, Gasull M. Number of persistent organic pollutants detected at high concentrations in a general population. Environ Int 2012;44:106–11.
[25] Porta M, Pumarega J, Gasull M, Lopez T. Concentration from endocrine disrupters of the general population at low and high concentrations. Vitam Horm 2014;94:167–92.
[26] Ngwa EN, Kengne A-P, Tiedeu-Atogho B, Moto-Mato E-P, Sobngwi E. Persistent organic pollutants as risk factors for type 2 diabetes. Diabetol & Metab Syndr 2015;7:41.
[27] Suarez-Lopez JR, Lee DH, Porta M, Steffes MW, Jacobs Jr DR. Persistent organic pollutants in young adults and changes in glucose related metabolism over a 23-years follow-up. Environ Res 2015;137:485–94.
[28] Dirinck EL, Dirtu AC, Govindan M, Covaci A, Van Gaal LF, Jorens PG. Exposure to persistent organic pollutants: relationship with abnormal glucose metabolism and visceral adiposity. Diabetes Care 2014;37:1951–8.
[29] Arrebola JP, Fernández MF, Martin-Olmedo P, Bonde JP, Martín-Rodriguez JL, Expósito J, et al. Historical exposure to persistent organic pollutants and risk of incident hypertension. Environ Res 2015;138:217–23.
[30] Goncharov A, Pavuk M, Foushee HR, Carpenter DO. Blood pressure in relation to concentrations of PCB congeners and chlorinated pesticides. Environ Health Perspect 2011;119:319–25.
[31] Arrebola JP, Pumarega J, Gasull M, Fernandez MF, Martin-Olmedo P, Molina-Molina JM, et al. Adipose tissue concentrations of persistent organic pollutants and prevalence of type 2 diabetes in adults from Southern Spain. Environ Res 2013;122:31–7.
[32] Wolff MS, Zeleniuch-Jacquotte A, Dubin N, Toniolo P. Risk of breast cancer and organochlorine exposure. Cancer Epidemiol Biomark Prev 2000;9:271–7.
[33] Kim KS, Hong NS, Jacobs Jr DR, Lee DH. Interaction between persistent organic pollutants and C-reactive protein in estimating insulin resistance among non-diabetic adults. J Prev Med Public Health 2012;45:62–9.
[34] Rantakokko P, Männistö V, Airaksinen R, Koponen J, Viluksela M, Kiviranta H, et al. Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a cohort study. Environ Health 2015;14:79.
[35] Gauthier M-S, Rabasa-Lhoret R, Prud'homme D, Karelis AD, Geng D, van Bavel B, et al. The metabolically healthy but obese phenotype is associated with lower plasma levels of persistent organic pollutants as compared to the metabolically abnormal obese phenotype. J Clin Endocrinol Metab 2014;99:E1061-6. Author Response to Ayotte et al., October 7, 2014. Available online: http://press.endocrine.org/e-letters/10.1210/jc.2013-3935.
[36] Kim KS, Lee YM, Kim SG, Lee IK, Lee HJ, Kim JH, et al. Associations of organochlorine pesticides and polychlorinated biphenyls in visceral vs. subcutaneous adipose tissue with type 2 diabetes and insulin resistance. Chemophere 2014;94:151–7.

Nutrition, Metabolism & Cardiovascular Diseases (2020) **30**, 448–458

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Effect of fatty fish or nut consumption on concentrations of persistent organic pollutants in overweight or obese men and women: A randomized controlled clinical trial

Sasa Dusanov [a,*], Mette Svendsen [a,d], Jerome Ruzzin [b], Hannu Kiviranta [c], Hanne L. Gulseth [a,e], Tor O. Klemsdal [a], Serena Tonstad [a]

[a] Section for Preventive Cardiology, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway
[b] Department of Biological Sciences, University of Bergen, Bergen, Norway
[c] National Institute for Health and Welfare, Department of Health Security, Kuopio, Finland
[d] Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo
[e] Department of Chronic Diseases and Ageing, Norwegian Instiute of Public Health, Oslo, Norway

Received 26 August 2019; received in revised form 12 November 2019; accepted 14 November 2019
Handling Editor: A. Siani
Available online 21 November 2019

KEYWORDS
POPs;
Metabolic syndrome;
Nutrition;
Fatty fish;
Nuts;
Overweight/obese

Abstract *Background and aims:* While excess energy intake and physical inactivity constitute the obvious causes of body fat accumulation, persistent organic pollutants (POPs) are novel factors that have been linked to cardiometabolic disorders. Major sources of POPs are animal fats including fatty fish. Given the putative protective effects of fish on cardiovascular disease, we explored whether high consumption of fatty fish increased serum concentrations of POPs.
Methods and results: Men and women aged 35–70 years with body mass index between 25 and 38 kg/m^2 and at least 1 cardiometabolic component were randomized to high intakes of fatty fish (mostly farmed salmon, ~630 g/week; n = 45), high intakes of nuts (~200 g/week; n = 42) or a control group following their usual diet but restricting fatty fish and nuts for 6 months (n = 44). Concentrations of 15 POPs (5 organochlorinated compounds, 2 dioxin-like polychlorinated biphenyls and 8 non-dioxin-like polychlorinated biphenyls) and cardiometabolic risk factors were measured at baseline and end of the study. Results showed that changes in concentrations of individual and classes of POPs did not differ between the dietary groups and controls ($p > 0.05$). Among cardiometabolic risk factors HDL-cholesterol increased in the fatty fish group compared to controls (+0.10 mmol/L, CI (0.05–0.20); $p = 0.005$) while no changes were observed in the group consuming nuts.
Conclusion: Fatty fish consumption for 6 months did not increase the serum concentrations of POPs in individuals with overweight or obesity and metabolic risk. While this finding appears reassuring regarding short-term intakes of farmed salmon, long term variations in POPs in adipose stores require further study.

* Corresponding author. Section for Preventive Cardiology, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, P.b. 4956 Nydalen, N-0424 Oslo, Norway.
E-mail address: sasdus@ous-hf.no (S. Dusanov).

https://doi.org/10.1016/j.numecd.2019.11.006

Background

Excess body fat and its adverse health consequences appear to be rapidly increasing in prevalence in most areas of the world. In the wake of the obesity epidemic, cardiometabolic disorders including metabolic syndrome and type 2 diabetes have resulted in major public health challenges [1]. In Norway, more than one-fifth of the adult population is obese, and the prevalence of abdominal obesity has increased disproportionately in women [2].

Major causes of obesity include excess energy intake and physical inactivity, but emerging evidence has linked environmental toxins, such as persistent organic pollutants (POPs) with the development of obesity, metabolic syndrome and type 2 diabetes [3]. POPs are lipophilic chemicals that bioaccumulate in adipose tissue of living organisms for decades. They are also measurable in serum and usually vary according to age and gender [4]. Associations between POPs stored in adipose tissue and circulating blood concentrations may differ according to the compound and previous exposure [5].

POPs appear to disrupt metabolic regulation, possibly leading to weight gain [6] and increased risk of metabolic syndrome [7]. Furthermore, a body of evidence links of POPs to type 2 diabetes. A meta-analysis that included 72 observational studies found sufficient evidence for a positive association of some POPs and type 2 diabetes, despite studies' heterogeneity [8]. Furthermore, POPs may contribute to cardiovascular disease (CVD). The PREDIMED-CANARIAS cohort study found that people at high risk for CVD showed a higher level of contamination by POPs [9]. Others found associations between several POPs and carotid atherosclerosis [10].

Populations are mainly exposed to POPs through the consumption of fatty animal food, especially of marine origin. A study found that the most consistent association between foods and concentrations of POPs was fish followed by dairy and meat, while vegetables, fruit and cereals were rarely related to POPs [11]. These results seem to indicate that modifying dietary patterns may be useful to decrease the burden of POPs and potentially the risk of CVD [9].

Among types of fish, evidence to date points to farmed Atlantic salmon as a dietary source of some POPs, because of contaminants in fish feed [12]. Burdens of POPs appear to be higher in farmed compared to wild salmon; furthermore farmed salmon from Europe has previously been shown to be more contaminated than farmed salmon from South and North America [13]. In recent years aquaculture feed has been increasingly based on plant oils, instead of feed of animal origin [14]. On the other hand, a recent study found lower levels of POPs in farmed compared to wild salmon [15]. Cooking methods also affect the concentrations of POPs. For example, cooking of raw salmon leads to significant loss of lipids and lower content of POPs per unit of weight [16].

The potential harms of POPs in farmed fish complicate the relations between eating fish and CVD. Including fish in the diet has been generally associated with good health outcomes. Recently a reduced risk of CVD was found among Mediterranean populations who reported consuming fish, particularly fatty fish $\geq$4 times weekly versus $<$2 times weekly [17]. However, a meta-analysis of 14 prospective studies found regional differences in the relation between fish consumption and all-cause and CVD mortality; studies conducted in Western countries showed a U-shaped curve, indicating that large intakes may be detrimental [18].

Another food often recommended to prevent CVD is nuts. Studies strongly support a protective association between nut consumption and CVD [19]. A review of meta-analyses that appeared in 2018 suggested that nuts may be associated with lower all-cause mortality and CVD and coronary heart disease mortality, while there was no association between nut consumption and type 2 diabetes [20]. The PREDIMED dietary trial provided experimental evidence to support the protective effect of nuts on CVD [21]. Nuts could be a food alternative to people that either do not tolerate or dislike fatty fish and would not be expected to increase POP concentrations, though of course nut allergy also affects subsets of the population.

Given this background, the primary aim of this study was to clarify the effects of eating fatty fish (predominantly salmon and mackerell) or nuts (a mixture of walnuts, hazelnuts and almonds) on concentrations of POPs that are typically found in fatty fish compared to avoidance of fatty fish and nuts. We studied overweight and obese men and women with high risk of developing cardiometabolic disturbances that may be caused by high intakes of POPs.

Methods

Men and women aged 35–70 years with body mass index (BMI) 25–38 kg/m^2 were recruited through advertisement in newspapers, through the Facebook page of Oslo.

University Hospital as well as from patient referrals to the Section of Preventive Cardiology, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway. The study was approved by the local Regional Ethics Committee (Approval number 2015/1930) and conducted according to the Declaration of Helsinki. All participants provided written informed consent before any procedures were performed. The study, with protocol description was registered at www.clinicaltrials.gov (NCT02589756). The first participant was included in January 2016 and the last 6-month follow-up was in October 2017.

Inclusion criteria included 1 component of metabolic syndrome in addition to waist circumference ($\geq$102 cm for men or $\geq$88 cm for women), i.e. blood pressure $\geq$130/85 mmHg or use of antihypertensive medication, fasting glucose $\geq$5.6 mmol/L, HDL cholesterol $\leq$1.3 mmol/L for women or $\leq$1.0 mmol/L for men, or triglycerides $\geq$1.7 mmol/L. Exclusion criteria were cigarette smoking, diabetes, allergy to or dislike of fish or nuts, chronic disease including cancer, gastrointestinal disease or CVD,

morbid obesity (BMI of ≥38 kg/m^2 with obesity-related health conditions or ≥40 kg/m^2 alone) and self-reported weight fluctuations (+/− 5 kg in the last 6 months), eating disorder, history of bariatric surgery, use of anti-obesity drugs or other drugs affecting body weight, such as anty-psychotics or glucocorticoids.

Study design

At the screening visit, medical history and demographic information were recorded, including educational level and alcohol consumption and a medical examination was performed. Participants were also asked to fill out a food frequency questionnaire (FFQ) as described further below. Inclusion and exclusion criteria were applied after blood test results were available (usually on the same day). Thereafter, participants started with a run-in period of 2 weeks where they were asked not to consume salmon, mackerel or other fatty fish or nuts to provide a dietary baseline. Follow-up visits were scheduled at biweekly intervals up to 3 months, and thereafter every 6 weeks up to 6 months, for a maximum of 10 visits in total. Blood samples, weight, waist and hip circumferences, and blood pressure were taken at screening, randomization and 6 months. At every visit study food was distributed to participants in the fish or nuts groups. Participants were examined by the medical physician (SD) and were offered dietary instruction given by the nutritionists. The dietary instructions were mainly about how to incorporate the intervention foods in the diet. All participants received a booklet with recipes according to their study group.

Randomization

Randomization was performed by a stratified sampling procedure with gender and BMI (grouped as 25 to <30 kg/m^2 and 30−38 kg/m^2) as the strata. A statistician prepared a computer-generated random number list. **The clinic study assistant** opened numbered and sealed envelopes consecutively with no exceptions.

Dietary interventions

Participants randomized to the fatty fish group were asked to consume 4 portions (fillets) of farmed salmon provided in frozen portions (a total of 500−560 g weekly) and 1 tin of mackerel in tomato sauce (110 g) weekly. The energy content of the total amount of fish was ∼1400 kcal/week. This group was asked to avoid eating nuts. Fish was provided free of charge for the 6 months.

Participants randomized to the nuts group were asked to consume ∼100 g walnuts, ∼50 g hazelnuts and ∼50 g almonds weekly, also providing ∼1400 kcal/week. This recommendation is similar in composition and amount to the nut-mix used in the PREDIMED intervention study [21]. Nuts were provided free of charge for the 6 months. This group was asked to avoid eating fatty fish. However, lean fish was allowed.

The control group consumed their usual diet, but was asked to avoid fatty fish and nuts. The control group was also allowed to eat lean fish. At the end of the project this group received a gift card of 1200 Norwegian kroner (approximately 125 euros) as compensation for not receiving food during the project.

Data collection of dietary intake

Collection of dietary intake was done using the FFQ developed in conjunction with the Norwegian MoBa study [22]. Data were collected at screening and at 6 months. The FFQ at screening reflected the habitual diet the past year prior to the start of the study, while the FFQ at 6 months reflected the participants' diet during the study period of the last 6 months. The FFQ consisted of 255 different food and drink items. The food items were divided into categories like "bread, crisp bread and crackers" or "hot meals", with following suitable subcategories to specify details on each particular food. Drinks, desserts and snacks were mapped in the same manner as other food items in the attempt to include all sources of energy. The frequency of the reported food items was given in either number per day, number per week or number per month, with the possibility to mark only 1 of the 3 frequency intervals. Portion sizes were given for units of bread (slices), liquids (cups/glasses) and fruit. In cases where they were not given, consumption frequencies were converted into food amounts (g/day) by considering standard Norwegian portion sizes for men and for women.

The participants were given instructions on how to fill out the form and to consider seasonal food such as for Christmas or summer, as a mean throughout the year. Food items consumed less than 6 or 12 times a year (in the screening or end of study FFQ, respectively) were not queried.

The FFQs were examined by trained nutritionists in order to ensure accuracy as far as possible. Corrections were made in collaboration with each individual participant. Data on food items and nutrient consumption were calculated using FoodCalc [23] and the Norwegian food composition table from 2003 [24]. The calculations were performed in the programs originally developed for this FFQ at Norwegian Institute of Public Health.

Checks of compliance with assigned foods were done at each visit by the dietitian/responsible physician by direct query as to whether all of the assigned food had been consumed. Participants in the group assigned to fatty fish reported full compliance with the assigned weekly amounts with the exception of 2 participants. One of these participants consumed 1 portion less weekly than the 4 weekly assigned portions due to prestudy established nausea and esophageal reflux while the other participant reported a pause of 2 weeks in fish intake during a

vacation after the 3-month visit but compensated for this hiatus by consuming the assigned amount of fatty fish for 2 extra weeks at the end of the study (total study duration for this participant was 6.5 months). After considering these deviations, the amount of consumed salmon and mackerell consumed by the fatty fish group was estimated at ~90 g/day (~630 g/week).

Participants in the group assigned to nut consumption also reported full compliance during the study period with the exception of one participant who reported consuming ~60% of the assigned nuts between visit 8 and 9. The amount of ingested nuts was estimated at ~30 g/day in the nuts group.

Clinical and laboratory procedures

Body weight was measured using the same calibrated digital scale to the nearest 0.1 kg. Waist circumference was measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Hip circumference was measured at the widest portion of the buttocks. Blood pressure was measured using the Omron 705IT (Omron HEALTHCARE, Kyoto, Japan) after the participant rested quietly in a sitting position for at least 5 min alone in a quiet room. The mean of 3 measurements spaced 1 min apart was calculated at screening, randomization, and 3 month and 6 month visits.

Blood samples were obtained following a minimum of a 10-hour fast. Analyses of blood samples were performed at Oslo University Hospital Clinical Chemistry Laboratory/Ullevål. Lipids were measured using enzymatic colorimetric methods, while apolipoprotein B was determined using an immunoturbidimetric method. Serum glucose was measured using hexokinase. HbA1c was measured using ion-exchange quantitative high performance liquid chromatography. C-reactive protein (CRP) was determined with a particle enhanced turbidimetric assay. C-peptide and insulin concentrations were analyzed at the hormone laboratory of OUS/Aker, using a non-competitive electro-chemiluminescence immunoassay (ECLIA) (Modular E170 Cobas e601 kit Roche Diagnostics). The Homeostasis Model Assessment insulin resistance index (HOMA-IR) was calculated to estimate insulin resistance [25].

To further estimate insulin sensitivity a euglycemic hyperinsulinemic clamp was performed at the Diabetes Research Laboratory at Oslo University Hospital Aker in a subset of participants, 10 subjects in the control and 10 patients in the fatty fish group at randomization and 6 months. The hyperinsulinemic euglycemic clamp was performed after an overnight fast. A fixed dose of insulin 40 $mU/m^2/min^{-1}$ was infused, and glucose 200 mg/mL was adjusted to maintain plasma glucose levels at 5.0 mmol/L for 150 min (euglycemia). Insulin sensitivity was reported as glucose infusion rate during the last 30 min of the clamp. Prior to the clamp, weight and fat-free mass were measured with a Tanita Body Composition Analyzer BC-418MA (Tokyo, Japan).

Assessment of circulating POPs concentrations

POPs were measured in 200 μl of serum at the National Institute for Health and Welfare, Kuopio, Finland. The following POPs were measured: polybrominated diphenylethers (PBDEs) 47, 99 and 153; polychlorinated biphenyls (PCBs) including dioxin-like (118, 156) and non-dioxin-like (74, 99, 138, 153, 170, 180, 183, 187) compounds; and organochlorine pesticides including dichlorodiphenyltrichloroethane (p,p′DDT), dichlorodiphenyldichloroethylene (p,p′DDE), hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH), pentachlorobenzene (PeCB), hexachlorobenzene, *trans*-nonachlor, and oxychlordane. The detection rate for PeCB, α-HCH, γ-HCH, oxychlordane and all PBDEs was <75% thus, these analytes were excluded from further statistical analyses.

A full description of the analytical method has been published previously [26]. Limits of quantification for POPs were 5–40 pg/ml. In each batch of samples a control serum sample from the National Institute of Standard and Technology, Standard Reference Material (SRM) 1958 for POPs was included (n = 13). An in-house produced low level control sample was prepared by 1–9 dilution of SRM 1958 with new born calf serum (NBCS) and also included in each batch of samples (n = 13). Average concentrations for POPs from SRM 1958 were 87–111% of the certified/indicative concentrations. Coefficients of variation (CVs) were 1.4–4.4% for SRM 1958 and 1.8–8.9% for diluted SRM 1958. The Environmental Health Unit participates to AMAP inter laboratory comparison (Ring Test for Persistent Organic Pollutants in human serum, National Institute of Public Health, Quebec, Canada) where 16 of the target POPs from 3 serum samples are reported for each round. During the last 2 years results from all samples for all POPs have been acceptable ($|z|<2$). Accuracy of the results for individual compounds from individual samples varied from 77–121% of the assigned values.

Assessment of POPs in fish samples

Four samples of farmed salmon from the producers suppling the fish in the study were analyzed at the National Institute for Health and Welfare, Kuopio, Finland for POPs content utilizing the same method used for serum samples.

Outcome measures

The primary outcome endpoint was changes in concentrations of POPs including organochlorinated pesticides (5 compounds), dioxin-like PCBs (2 compounds) and non-dioxin-like PCBs (8 compounds). Secondary outcomes were changes in weight, BMI, waist circumference, blood pressure and heart rate, and other cardiometabolic risk factors including concentrations of total cholesterol, HDL- and LDL-cholesterol, triglycerides, apolipoprotein B, glucose and HbA1c as well as C-reactive protein, insulin and C-peptide, HOMA-IR and clamp.

Statistical analysis

Power calculation was attempted, however, given the lack of previous data was exploratory. Differences in hexachlorobenzene (HCB) between the highest and lowest tertile were about 3-fold in the Nurses' Health Study, while differences in total polychlorinated biphenyls (PCBs) between the highest and lower tertile were about 4-fold [27]. Another study showed about a doubling of levels between the highest and lowest quartiles [28]. In a 6-month period a possibly clinically relevant change in POPs may be a 15–20% increase – this is also the difference in PCBs between representative and high consumers in Norway [29]. Based on the median HCB level in the Nurses' Health Study [27] of about 30 ng/g lipids, a difference of a mean of 36 ng/g lipids between groups with a standard deviation of about 9 ng/g lipids would require 36 participants in each group, with power set at 80% and alpha set at 5%. Our intention was to include 40 participants in each group to allow for dropouts for a total of 120 participants. 1 participant in the nut group had β-HCH and p,p'DDE values that were outliers (4228, 7071 pg/ml, respectively). These values were removed but no substantial changes were seen in results after this exclusion.

Analyses followed the intent-to-treat principle with the last value carried forward for dropouts, with additional complementary analyses of the per protocol population. These analyses did not differ substantially, and the intent-to-treat analyses are presented. Independent Student's t-test was performed comparing fatty fish group and nut group with controls. Variables that were not normally distributed were presented as median and 25th and 75th percentile and changes were analyzed using the Mann–Whitney test. Data was analyzed using IBM SPSS Statistics for Windows version 21 (SPSS Inc., Chicago, IL). The significance level was set at $p < 0.05$.

Results

Of the total of 131 participants (56 men and 75 women) randomized, 120 completed the study (Fig. 1). Age ranged between 37 and 69 years, and BMI ranged between 25.5 and 38.2 kg/m^2. Screening characteristics, medication use and laboratory analyses are presented in Table 1. Antihypertensive medications were adjusted by the study physician or the participant's general practitioner during the study in 12 participants. Of these, 4 participants changed dosage (3 increased dosage - 1 in the nut group, 2 in the control group) and 1 participant in the fatty fish group reduced dosage. Two stopped medication (1 in the fatty fish group and 1 in the nut group). One participant in the fatty fish group started medication and 5 changed type of anti-hypertensive treatment (3 in the nut and 2 in the control groups, respectively). In addition, 1 participant in the control group started statin treatment.

Weight, BMI, waist and hip circumferences, systolic and diastolic blood pressure and heart rate remained unchanged and did not differ between the 3 groups (Table 2). Among cardiometabolic risk markers only HDL-cholesterol increased in fatty fish group in comparison to controls (+0.1 mmol/L, CI (0.05–0.2), $p = 0.005$). Other markers, including insulin, HOMA-IR index and c-peptide did not differ between groups. Change in glucose infusion rate did not differ between fatty fish group and controls ($p = 0.7$, data not shown).

Table 3 shows dietary intakes at screening and 6 months. In the group assigned to fatty fish, the energy percentage from protein increased while percentages from

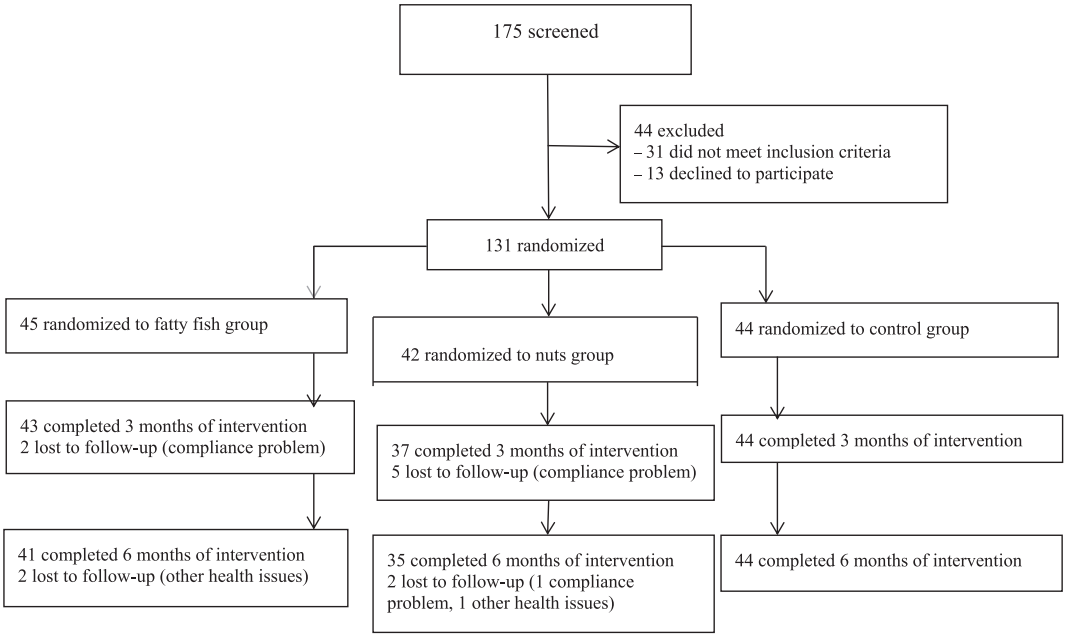


Figure 1 Consolidated Standards of Reporting Trials (CONSORT) flow diagram for participants' allocation into study arms.

Table 1 Screening characteristics. Mean (SD) values or percentages are shown, except for alcohol consumption, shown as median (25th, 75th percentile).

	Fatty fish diet (n = 45)	Nuts diet (n = 42)	Controls (n = 44)
Females, n (%)	26 (57.8)	24 (57.1)	25 (56.8)
Age, years	55.9 (6.6)	58.0 (5.2)	55.0 (6.9)
Education attained			
Primary school, n (%)	1 (2.2)	–	1 (2.3)
High school, n (%)	17 (37.8)	9 (21.4)	11 (25.0)
University, n (%)	27 (60.0)	33 (78.6)	32 (72.7)
Alcohol consumption (units/week)	2 (0, 5)	3 (1, 7)	3 (0, 5)
Body weight, kg	94.7 (13.4)	94.4 (13.5)	92.9 (15.1)
BMI, kg/m^2	31.1 (2.8)	31.7 (3.1)	31.2 (2.9)
Waist circumference males, cm	116 (10)	114 (9)	112 (7)
Waist circumference females, cm	108 (8)	109 (7)	108 (8)
Systolic blood pressure, mmHg	136 (15)	137 (13)	134 (13)
Diastolic blood pressure, mmHg	85 (10)	86 (9)	83 (7)
Heart rate/minute	68 (9)	70 (9)	66 (9)
Medications			
Use of antiplatelet agents, n (%)	6 (13.3)	5 (11.9)	3 (6.8)
Use of antihypertensives, n (%)	18 (40.0)	20 (47.6)	15 (34.1)
Use of lipid lowering drugs, n (%)	9 (20.0)	7 (16.7)	7 (15.9)
Laboratory values, fasting			
Total cholesterol, mmol/L	5.5 (1.1)	5.6 (1.1)	5.7 (1.2)
HDL-cholesterol, mmol/L	1.4 (0.3)	1.5 (0.4)	1.5 (0.4)
LDL-cholesterol, mmol/L	3.7 (0.9)	3.7 (1.0)	3.8 (1.1)
Triglycerides, mmol/L	1.5 (0.8)	1.4 (0.6)	1.5 (1.0)
Glucose, mmol/L	5.7 (0.5)	5.6 (0.5)	5.8 (0.7)
Metabolic syndrome, n (%)	31 (69)	19 (45)	22 (50)

carbohydrates and fat decreased in the fatty fish group compared to controls (Table 3). On the other hand, the energy percentages of total, mono- and polyunsaturated fat increased and the energy percentage from carbohydrates decreased in the group assigned to nuts compared to controls.

Neither of the intervention groups showed significant difference in the circulating concentrations of selected POPs (organochlorine pesticides including HCB, β-HCH, *trans*-nonachlor p,p'DDT, p,p'DDE, dioxin-like PCBs (118, 156) and non-dioxin-like PCBs (74, 99, 138, 153, 170, 180, 183, 187)) compared to controls after 6 months (Table 4).

Analyses of POPs concentrations in fish samples are presented in Table 5.

Discussion

This randomized controlled clinical trial found no change in circulating concentrations of 15 POPs during 6 months of high dietary intakes of fatty fish compared to avoidance of fatty fish in the diet among persons at risk of cardiometabolic disorders. In addition to comparison with a control group avoiding fatty fish, we included a group consuming nuts and avoiding fatty fish. Likewise no differences were found between the group consuming fatty fish compared to nuts group (data not shown). Cardiometabolic risk markers did not change in the groups consuming fatty fish or nuts compared to the group asked to avoid both food groups, with the exception of concentrations of HDL-cholesterol that increased in the group consuming fatty fish.

The study used a randomized controlled design to avoid bias and a randomization procedure with stratification for gender and BMI. Participants were approximately equally divided according to gender and selected in regard to the presence of overweight or obesity and at least 1 other cardiometabolic risk factor. They thus represented a sample of the population most likely to show possible cardiometabolic risks of eating fatty fish. During the trial there was a low level of dropouts overall (8%). Furthermore, participants showed good compliance with the assigned dietary group, as shown by compliance checks at each visit and the FFQ results.

We are unaware of any published similar randomized interventional studies that explored POPs concentrations in overweight and obese individuals in regard to fatty fish intakes. However, these types of studies may not be ideal to study the effect of diet on POPs as the main factor determining changes in serum concentrations of POPs during a relative short period of 6 months would be the dynamics of POPs storage and release. While body weight was carefully monitored and stayed stable, a number of factors may influence these dynamics mostly over longer periods of time.

The POPs that we measured are classified as organochlorine pesticides and dioxin-like and non-dioxin-like PCBs. These POPs have been connected to cardiometabolic disturbances both in cross-sectional and prospective studies [30]. We recently published a cross-sectional study showing that high concentrations of the same organochlorine pesticides and dioxin-like and non-dioxin-like PCBs, as measured in the current study, more than

Table 2 Changes in clinical characteristics and cardiometabolic markers between baseline and six months. Mean (SD) values are shown, except for C-reactive protein, insulin and c-peptide concentrations for which median (25th, 75th percentile) values are shown.

	Fatty fish diet (n = 45)				Nuts diet (n = 42)				Controls (n = 44)
	Baseline	6 months[a]	Change	P[b]	Baseline	6 months[a]	Change	P[b]	Change
Weight, kg	95.0 (13.9)	94.7 (14.4)	−0.3 (3.3)	0.4	94.3 (14.1)	94.8 (14.1)	0.4 (2.6)	0.7	0.2 (2.4)
BMI, kg/m^2	31.1 (2.8)	31.0 (3.2)	−0.1 (1.0)	0.4	31.5 (2.8)	31.7 (2.9)	0.2 (1.0)	0.5	0.1 (0.8)
WC[d] male, cm	116 (9)	116 (10)	0.5 (3.2)	0.9	114 (9)	115 (6)	0.9 (3.2)	0.7	0.4 (4.0)
WC female, cm	108 (8)	110 (9)	0.5 (3.4)	0.6	109 (8)	109 (9)	1.0 (4.0)	0.4	−0.1 (4.5)
SBP, mmHg	133 (12)	130 (12)	−4 (11)	1.0	135 (14)	131 (16)	−4 (9)	1.0	−4 (14)
DBP, mmHg	83 (7)	81 (7)	−1 (7)	0.8	85 (9)	83 (10)	−2 (5)	1.0	−2 (6)
Heart rate/min	68 (10)	67 (10)	−1 (7)	0.4	71 (11)	71 (8)	0 (8)	0.8	0 (8)
Cardiometabolic markers									
Total cholesterol mmol/L	5.3 (1.0)	5.4 (1.1)	0.0 (0.5)	0.01	5.5 (1.0)	5.5 (1.1)	0.0 (0.5)	0.1	−0.3 (0.5)
HDL-cholesterol, mmol/L	1.4 (0.3)	1.5 (0.4)	0.1 (0.2)	0.005	1.5 (0.3)	1.5 (0.4)	0.0 (0.1)	0.7	0.0 (0.2)
LDL-cholesterol, mmol/L	3.5 (0.8)	3.4 (0.9)	−0.1 (0.5)	0.3	3.7 (0.9)	3.7 (0.9)	0.0 (0.5)	0.06	−0.2 (0.4)
Triglycerides, mmol/L	1.6 (1.1)	1.5 (1.5)	−0.1 (0.7)	0.7	1.5 (0.6)	1.6 (0.8)	0.1 (0.5)	0.2	−0.1 (0.6)
Apolipoprotein B, g/L	1.1 (0.2)	1.1 (0.2)	0.0 (0.1)	0.1	1.1 (0.2)	1.1 (0.3)	0.0 (0.2)	0.2	0.0 (0.2)
Glucose mmol/L	5.6 (0.5)	5.6 (0.4)	0.0 (0.4)	0.3	5.7 (0.4)	5.8 (0.5)	0.1 (0.4)	0.8	0.1 (0.4)
HbA1c, %	5.3 (0.3)	5.6 (0.4)	0.0 (0.1)	0.1	5.3 (0.3)	5.3 (0.4)	0.0 (0.2)	0.6	0.0 (0.2)
C-reactive protein, mg/L	1.7 (0.8–3.8)	1.6 (0.7–3.8)	0.0 (−0.5–0.4)	0.3	1.6 (1.0–3.5)	2.4 (0.9–4.9)	0.0 (−0.4–0.9)	0.3	0.4 (−0.1–0.9)
Insulin, pmol/L[c]	68 (51, 114)	85 (58, 127)	8 (−15, 25)	0.7	75 (55, 95)	83 (62, 116)	4 (−22, 21)	0.9	3 (−13, 21)
C-peptide, pmol/L	887 (665, 1173)	990 (847, 1243)	91 (−50, 222)	0.08	893 (807, 1052)	959 (802, 1209)	25 (−133, 188)	0.6	4 (−88, 93)
HOMA-IR index	1.8 (0.9)	1.8 (1.4)	0.0 (1.1)	0.7	1.9 (0.8)	1.8 (1.0)	−0.1 (0.7)	0.8	0.0 (0.8)

[a] Values for 6 and 5 participants, respectively, were carried forward from baseline in the fish and nut groups.
[b] T-test compared each of fish and nut groups to controls.
[c] Insulin and c-peptide values (5 missing in fatty fish group, 8 missing in nuts group, 2 missing in controls) Non-parametric Mann–Whitney test compared each of fish and nut groups with controls.
[d] WC waist circumference.

Table 3 Changes in dietary intake between baseline and six months. Mean (SD) values are shown.

	Fatty fish diet (n = 36)				Nuts diet (n = 30)				Controls (n = 37)
	Baseline	6 months	Change	P[a]	Baseline	6 months[a]	Change	P[a]	Change
Study foods									
Fatty fish, g/day	31.6 (20.2)	90.4 (4.3)	58.7 (19.5)	<0.001	33.4 (31.7)	1.0 (2.7)	−32.4 (32.1)	0.8	−30.4 (20.7)
Nuts, g/day	9.6 (14.1)	0.0 (0.2)	−9.6 (14.1)	0.1	6.9 (7.6)	30.0 (−)	23.1 (7.6)	<0.001	−5.2 (7.5)
Macronutrients									
Energy, kJ/day	8866 (2830)	8040 (2334)	−625 (2108)	0.5	8872 (2477)	8598 (1998)	−275 (2149)	0.9	−331 (1745)
Energy, kcal/day	2057 (666)	1914 (556)	−143 (492)	0.5	2110 (588)	2047 (474)	−63 (511)	0.9	−76 (415)
Protein, E%	16.3 (1.9)	18.3 (1.9)	2.0 (2.0)	<0.001	17.0 (2.3)	16.9 (2.2)	−0.1 (2.2)	0.6	0.2 (2.1)
Carbohydrates, E%	44.3 (7.4)	42.5 (6.9)	−1.7 (4.6)	<0.001	46.0 (5.3)	42.5 (7.2)	−3.5 (5.3)	<0.0001	2.6 (4.7)
Fat, E%	36.6 (6.6)	36.2 (5.1)	−0.4 (4.7)	0.008	33.4 (4.1)	37.0 (5.5)	3.6 (5.1)	<0.0001	−3.3 (4.6)
Saturated fat, E%	13.1 (2.0)	12.7 (2.2)	−0.6 (1.8)	0.4	12.1 (2.2)	11.7 (2.2)	−0.3 (2.3)	0.5	0.0 (1.8)
Monounsaturated fat, E%	13.1 (3.4)	12.1 (2.1)	−1.0 (2.4)	0.1	11.7 (1.7)	13.9 (2.4)	2.2 (2.3)	<0.0001	−1.9 (2.7)
Polyunsaturated fat, E%	6.7 (1.9)	6.4 (1.6)	−0.3 (1.9)	0.054	6.1 (1.1)	7.9 (1.4)	1.9 (1.1)	<0.0001	−1.0 (1.3)
Alcohol, E%	2.8 (3.3)	2.7 (3.8)	−0.1 (2.2)	0.3	3.5 (3.0)	3.3 (2.9)	−0.2 (1.8)	0.2	0.4 (2.1)
Fiber, E%	2.8 (0.6)	2.7 (0.7)	−0.1 (0.5)	0.4	2.8 (0.6)	2.9 (0.6)	0.1 (0.5)	0.6	0.0 (0.8)

[a] T-test used to compare each of fatty fish or nut diets versus controls.

Table 4 Changes in POPs between baseline and six months. Mean (SD) values are shown, for change mean (CI) values are shown.

	Fatty fish diet (n = 45)			Nuts diet (n = 42)			Controls (n = 44)
	Baseline	6 months[a]	Change	Baseline	6 months[a]	Change	Change
Organochlorinated compounds (pg/ml)							
HCB	88.4 (33.9)	87.2 (32.8)	−1.2 (−5.4−3.1)	90.6 (32.3)	89.5 (32.5)	−1.0 (−4.3−2.2)	−3.5 (−7.4−0.4)
β-HCH	16.6 (12.6)	16.4 (11.0)	−0.2 (−1.5−1.0)	27.4 (46.1)	26.7 (43.1)	−0.7 (−2.5−1.1)	−1.3 (−2.4−0.1)
trans-Nonachlor	42.5 (26.5)	40.6 (26.1)	−1.9 (−4.8−1,0)	48.9 (38.6)	49.4 (38.3)	0.5 (−3.6−4.7)	−3.2 (−6.2−0.2)
p,p'DDT	8.2 (1.3)	8.2 (1.0)	0.0 (−0.6−0.5)	10.2 (7.8)	9.9 (7.0)	−0.3 (−0.8−0.2)	−0.5 (−1.1−0.1)
p,p'DDE	443.9 (394.1)	414.2 (372.0)	−29.8 (−73.6−14.0)	463.8 (298.5)	476.8 (324.9)	13.0 (−15.7−41.6)	−16.9 (−43.9−10.1)
Dioxin-like polychlorinated biphenyls (pg/ml)							
PCB-118	50.1 (35.1)	46.6 (31.6)	−3.5 (−9.0−2.0)	53.1 (30.3)	52.8 (30.1)	−0.3 (−2.5−1.9)	−1.8 (−4.6−1.1)
PCB-156	36.1 (17.9)	34.7 (18.0)	−1.4 (−3.9−1.2)	37.1 (17.5)	38.2 (18.3)	1.1 (−1.9−4.1)	−1.2 (−3.4−1.0)
Nondioxin-like polychlorinated biphenyls (pg/ml)							
PCB-74	19.4 (13.1)	19.0 (13.4)	−0.4 (−2.1−1.3)	19.9 (14.9)	19.6 (14.7)	−0.3 (−1.3−0.7)	−0.8 (−1.8−0.1)
PCB-99	23.8 (16.8)	22.7 (15.6)	−1.1 (−2.7−0.5)	28.2 (20.9)	28.1 (21.0)	−0.1 (−1.7−1.4)	−1.1 (−2.3−0.1)
PCB-138	167.3 (90.3)	159.4 (84.9)	−8.0 (−20.3−4.4)	177.8 (89.7)	179.9 (91.8)	2.1 (−10.7−15.0)	−6.0 (−15.1−3.0)
PCB-153	294.7 (148.0)	280.7 (142.1)	−14.0 (34.5−6.4)	305.6 (139.1)	313.1 (145.7)	7.5 (−15.2−30.2)	−8.9 (−25.7−7.9)
PCB-170	103.4 (51.0)	98.9 (50.7)	−4.5 (−11.6−2.6)	102.1 (45.9)	105.2 (49.1)	3.1 (5.2−11.4)	−3.2 (−8.9−2.5)
PCB-180	234.6 (113.0)	225.0 (113.2)	−9.7 (−24.6−5.2)	236.0 (106.0)	242.8 (114.4)	6.8 (−11.8−25.3)	−6.1 (−19.0−6.7)
PCB-183	17.4 (10.3)	16.4 (9.4)	−1.0 (−2.4−0.5)	18.5 (10.1)	18.9 (10.7)	0.4 (−1.0−1.8)	−0.8 (−1.8−0.3)
PCB-187	60.2 (32.0)	57.9 (31.7)	−2.3 (−7.0−2.3)	62.1 (28.2)	62.7 (29.2)	0.6 (−3.8−5.0)	−2.0 (−5.5−1.6)

HCB =Hexachlorobenzene, HCH-beta = β-hexachlorocyclohexane, p,p'DDT = dichlorodiphenyltrichloroethane, p,p'DDE = Dichlorodiphenyldichloroethylene, PCB = polychlorinated biphenyl.

[a] Values for 4 and 6 participants in the fish and nut groups, respectively, were carried forward from baseline.

double the odds ratio for metabolic syndrome in obese people [31].

POPs have been associated with a range of cardiometabolic risk factors in particular insulin-resistance and related disorders [32]. We previously showed associations between POPs and cardiometabolic risk factors [31], giving the background for conducting the present study. However, a cross-sectional study conducted among Inuits living in Greenland found that POPs may adversely affect insulin secretion [33], giving support to the notion that POPs may be more prominent in the development of beta-cell dysfunction-type diabetes rather than the insulin-resistance type [34]. Insulin secretion was not studied in the current trial. While most studies did not identify a specific compound, in one study p,p'DDE concentrations were elevated in patients with diabetes [35]. In the current study we did not find changes in HOMA-IR index or in the glucose infusion rate between participants in the fatty fish group versus controls, however, only 10 individuals from each group underwent the clamp procedure.

Table 5 POPs concentrations in fatty fish samples (n = 4). Mean (range) values are shown.

Organochlorinated pesticides (pg/g)	
HCB	781 (386−1034)
β-HCH	65 (25−87)
trans-Nonachlor	485 (220−656)
p,p'DDT	390 (240−543)
p,p'DDE	1926 (855−2632)
Dioxin-like polychlorinated biphenyls (pg/g)	
PCB-118	354 (155−474)
PCB-156	38 (14−52)
Nondioxin-like polychlorinated biphenyls (pg/g)	
PCB-74	63 (27−84)
PCB-99	263 (108−362)
PCB-138	736 (313−981)
PCB-153	917 (387−1273)
PCB-170	108 (40−178)
PCB-180	237 (92−377)
PCB-183	67 (25−113)
PCB-187	284 (117−445)

In contrast to the putative detrimental effects of POPs on cardiometabolic risks, fish oils (or fatty fish) may improve cardiometabolic risk factors. In line with our observation of an increase in HDL-cholesterol in the fatty fish group, systematic review and meta-analysis concluded that consuming fish improved HDL-cholesterol and triglyceride levels [36]. A study conducted in volunteers with impaired glucose metabolism found that fatty fish increased HDL particle diameter and concentrations of lipid components in HDL, possibly boosting the anti-atherogenic properties of HDL [37]. Also, another study found that fresh fish was superior to omega-3 supplements in increasing HDL-cholesterol and positively modifying lipid profiles [38]. It is possible that increase in HDL-cholesterol in the fatty fish compared to the group consuming nuts reflects the lack of fatty fish in the diet of the group consuming nuts. We did not find a reduction in triglyceride concentrations in the fatty fish group, perhaps because participants' baseline levels were only mildly elevated.

Uncertainty remains regarding the associations between fish, n-3 fatty acids and their effects on CVD. If POPs and other pollutants may impair the nutritional benefits of fish [39] using fish oil supplements may be an alternative. A recent meta-analysis attributed the benefits of eating fatty fish as being due to their n-3 fatty acid content [40]. However, it appears that evidence of benefits from n-3 fatty acids supplementation has diminished over time, possibly due to better pharmacological prevention and

treatment of patients with CVD [41]. The type of n-3 fatty acid may play a role. A recent study showed that ingestion of a total of 4 g eicosapentaenoic acid daily in individuals with elevated triglyceride levels lowered cardiovascular death compared with placebo, despite use of statins by all participants [42]. Generally, the majority of dietary factors that may reduce CVD are food groups, rather than isolated nutrients [43], both because of the balance of micronutrients and other substances in foods and substitution of good foods for other, less healthy choices. There is evidence that lean fish could also play a protective role due to other useful nutrients [40].

We found no increase in POPs in the nuts group compared to controls in the current study. A previously cited meta-analysis confirms multiple observations that nut consumption may lower all-cause mortality and CVD [20]. A review from 2018 based on cohort and interventional trials indicates that some nutrients that are richly found in nuts such as L-arginine, some minerals, phytosterols and unsaturated fatty acids could be linked to beneficial health effects of nuts [44]. For example, a review of meta-analyses found that nuts lowered LDL cholesterol [20], a finding that did not attain statistical significance in the current study. A randomized crossover trial found that both fatty fish and walnuts lowered cholesterol and triglyceride concentrations in normal to mildly hyperlipidemic individuals [45]. However, one study showed that nuts and seeds could be rich in PCBs, while other contaminants typical for animal foods are not present in nuts in significant concentrations [46]. An interventional study that compared a hypocaloric vegetarian and conventional diet did not find any reduction in concentrations of POPs in the vegetarian group, compared to conventional diet, possibly due to mobilization of fat stores in response to a decreased calorie intake [47].

Limitations

While the short study period may not be optimal to show changes in serum POPs concentrations, some smaller studies have found changes in serum POPs after short times of follow-up. For example one study reported reduction of POPs after 2 months of vitamin C supplementation [48] and likewise reductions were reported after one year of supplementation with olestra [49]. The validity of such studies is not clear.

A further limitation relates to possible recall bias while filling out the FFQ, as the FFQ covered a year at inclusion and the past 6 months at the end of the study. Notably, the food composition of salmon has been changed from marine-based diet in the early 1990s to a 70% plant based diet at present and that resulted in n-3 fatty acid content of the fish [50]. Contaminants like polycyclic aromatic hydrocarbons (PAHs), which are omnipresent in vegetable oils, are used in aquaculture today [51]. Some findings indicate that development in feed formulations may reduce traditional POPs in salmon, but may increase others, such as PAHs, normally not connected to salmon [52], however, PAH concentrations were not analyzed in the current trial. We did not analyze the content of POPs in the nuts used.

We did not analyze body adipose tissue samples for changes in contamination by POPs. In a previous small and nonrandomized study consumption of 380 g of farmed salmon weekly did not increase concentrations of HCB, p,p'DDE, sum of PCBs in plasma or adipose tissue samples indicating that steady-state levels of POPs were not affected by fatty fish consumption [53].

In the current study protein intakes unavoidably increased modestly in the fatty fish group. A recent study conducted in mice found that the accumulation of POPs in adipose tissue and liver was affected by macronutrient intake, and not the total intake of POPs [54]. A high-fat and high-protein diet resulted in lower deposition of POPs in the adipose tissue and liver than did a low-fat and high-carbohydrate diet. These findings may suggest the importance of controlling macronutrients in dietary studies of POPs. Absolute contents of POPs in diet are not critical in determining the body burden.

Finally, as discussed recently [34] there are disadvantages to RCTs in the study of effects of diet on serum POP concentrations due to fluctuations and non-linear responses. However, an observational study that aimed to study whether fatty fish increased serum POP concentrations would need to elicit dietary data from food frequency questionnaires and more or less reliable data on the content of POPs in the diet as measured many years earlier could be fraught with limitations [34].

Comparisons of POPs content of salmon

We compared results of our analyses of POPs in fish samples from manufacturers who provided fish in the present study (Table 5) to values published by the Norwegian Institute of Marine Research [55]. We found similar values for HCB (mean, 0.78 μg/kg compared to 0.95 μ/kg, respectively), while β-HCH (mean 0.06 μg/kg compared to 0.16 μg/kg) and p,p'DDT (mean 0.4 μg/kg compared to 5.70 μg/kg) values were lower in the fish samples. Analyses for other POPs were not available [55].

Conclusion

Our main findings did not show changes in circulating POP concentrations in persons with overweight or obesity and at least 1 metabolic risk factor after 6 months of eating high amounts of fatty fish (>600 g/week) or nuts compared to avoiding fatty fish and nuts. In addition, intake of fatty fish or nuts was not associated with improved cardiometabolic risks.

Funding

Funding was provided from the Extrastiftelsen via the Norwegian Health Association. NorgesGruppen provided fish, nuts and the gift cards free of charge to participants. The funding organizations were not represented in the writing group and had no role in study design, data

collection, analysis, decision to publish or preparation of the manuscript.

Declaration of Competing Interest

The authors have no conflict of interest to declare. All authors had access to the data and have seen and approved the final submitted manuscript.

Acknowledgements

We thank nutritionists Synnøve Thon and Gabriele Foss for help with dietary counseling and data collection. Our patient secretaries Tonje Berg, Lisa Flakk and Anne Britt Foss are thanked for administrative support and the cardiology speciality nurses Ragnhild Kleve and Lise Bergengen are thanked for blood sampling and project support. We also thank the nurses Åse Halsne, Gro Boezelijn and Gøril Vinje at the Diabetes Research Laboratory, Margaretha Alice Haugen at the Norwegian Public Health Institute who calculated dietary intake from the FFQ. Mrs Tuula Rissanen and Mrs Arja Moilanen are acknowledged for performing the POP analyses in serum and fish samples.

References

[1] Engin A. The definition and prevalence of obesity and metabolic syndrome. Adv Exp Med Biol 2017;960:1–17.
[2] Midthjell K, Lee CM, Langhammer A, Krokstad S, Holmen TL, Hveem K, et al. Trends in overweight and obesity over 22 years in a large adult population: the HUNT study, Norway. Clin Obes 2013; 3(1–2):12–20.
[3] Ngwa EN, Kengne AP, Tiedeu-Atogho B, Mofo-Mato EP, Sobngwi E. Persistent organic pollutants as risk factors for type 2 diabetes. Diabetol Metab Syndrome 2015;7:41.
[4] Battermann SA, Chemyak S, Su FC. Measurement and comparison of organic compound concentrations in plasma, whole blood and dried blood spot samples. Front Genet 2016:7–64.
[5] De Roos AJ, Ulrich CM, Sjodin A, McTierman A. Adiposity, body composition, and weight change in relation to organochlorine pollutant plasma concentrations. J Expo Sci Environ Epidemiol 2012;22:617–24.
[6] Lyche JL, Nourizadeh- Lillabadi R, Karlsson C, Stavik V, Berg V, Skåre JU, et al. Natural mixtures of POPs affected body weight gain and induced transcription of genes involved in weight regulation and insulin signaling. Aquat Toxicol 2011;102(3–4):197–204.
[7] Gasull M, Castell C, Pallarès N, Miret C, Pumarega J, Te Llez-Plaza M, et al. Blood concentrations of persistent organic pollutants and unhealthy metabolic phenotypes in normal-weight, overweight, and obese individuals. Am J Epidemiol 2018;187(3):494–506.
[8] Taylor KW, Novak RF, Anderson HA, Birnbaum LS, Blystone C, Devito M, et al. Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: a national toxicology program workshop review. Environ Health Perspect 2013;121(7):774–83.
[9] Henriquez-Hernandez LA, Luzardo OP, Zumbado M, Serra-Majem L, Valeron PF, Camacho M, et al. Determinants of increasing serum POPs in a population at high risk for cardiovascular disease. Results from the PREDIMED-CANARIAS study. Environ Res 2017:477–84.
[10] Lind PM, Van Bavel B, Salihovic S, Lind L. Circulating levels of persistent organic pollutants (POPs) and carotid atherosclerosis in the elderly. Environ Health Perspect 2012;120(1):38–43.
[11] Schecter A, Colacino J, Haffner D, Patel K, Opel M, Papke O, et al. Perfluorinated compounds, polychlorinated biphenyls, and organochlorine pesticide contamination in composite food samples from Dallas, Texas, USA. Environ Health Perspect 2010;118: 796–802.
[12] Crinion WJ. The role of persistent organic pollutants in the worldwide epidemic of type 2 diabetes mellitus and the possible connection to farmed Atlantic salmon (Salmo salar). Altern Med Rev 2011;16(4):301–13.
[13] Hites RA, Foran JA, Carpenter DO, Hamilton MC, Knuth BA, Schwager SJ, et al. Global assessment of organic contaminants in farmed salmon. Science 2004;303:226–9.
[14] https://www.forskningsradet.no/en/Newsarticle/Raising_efficiency_sustainability_in_salmon_farming/1253918856119?lang=en.
[15] Lundebye AK, Lock EJ, Rasinger JD, Nøstbakken OJ, Hannisdal R, Karlsbakk E, et al. Lower levels of persistent organic pollutants, metals and the marine omega 3-fatty acid DHA in farmed compared to wild Atlantic salmon (Salmo salar). Environ Res 2017; 155:49–59.
[16] Bayen S, Barlow P, Lee HK, Obbard JP. Effect of cooking on the loss of persistent organic pollutants from salmon. J Toxicol Environ Health 2005;68(4):253–65.
[17] Bonaccio M, Ruggiero E, Di Castelnuovo A, Constanzo S, Persichillo M, De Curtis A, et al. Fish intake is associated with lower cardiovascular risk in a Mediterranean population: prospective results from the Moli-sani study. Nutr Metab Cardiovasc Dis 2017;27(10):865–73.
[18] Jayedi A, Shab-Bidar S, Eimeri S, Djafarian K. Fish consumption and risk of all-cause and cardiovascular mortality: a dose-response meta-analysis of prospective observational studies. Public Health Nutr 2018;21(7):1297–306.
[19] Afshin A, Micha R, Khatibzadeh S, Mozafarrian D. Consumption of nuts and legumes and risk of incident ischemic heart disease, stroke, and diabetes: a systematic review and meta-analysis. Am J Clin Nutr 2014;100:278–88.
[20] Kim Y, Keogh J, Clifton PM. Nuts and cardio-metabolic disease: a review of meta-analyses. Nutrients 2018;10(12).
[21] Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. N Engl J Med 2018;378(25):e34.
[22] Magnus P, Irgens LM, Haug K, Nystad W, Skjaerven R, Stoltenberg C, et al. Cohort profile: the Norwegian mother and child cohort study (MoBa). Int J Epidemiol 2006;35(5):1146–50.
[23] Lauritzen, J: FoodCalc. Data program accessible on internet, October 2002: from the project diet, cancer and health project at the Danish Cancer Society
[24] Mattilsynet AfeU. Den norske matvaretabellen. cited 2017 02.02.17]. Available from: www.matvaretabellen.no2016. [Accessed 19 May 2016]. http://www.matvaretabellen.no/.
[25] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487–95.
[26] Koponen J, Rantakokko P, Airaksinen R, Kiviranta H. Determination of selected perfluorinated alkyl acids and persistent organic pollutants from a small volume human serum sample relevant for epidemiological studies. J Chromatogr A 2013;1309:48–55.
[27] Wu H, Bertrand KA, Choi AL, Hu FB, Laden F, Grandjean P, et al. Persistent organic pollutants and type 2 diabetes: a prospective analysis in the nurses' health study and meta-analysis. Environ Health Perspect 2013;121:153–61.
[28] Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs Jr DR. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. PLoS One 2011;6:e15977.
[29] Kvalem HE, Knutsen HK, Thomsen C, Haugen M, Stigum H, Branysaeter AL, et al. Role of dietary patterns for dioxin and PCB exposure. Mol Nutr Food Res 2009;53:1438–51.
[30] Shi H, Jan J, Hardesty JE, Falkner KC, Prough RA, Balamurugan AN, et al. Polychlorinated biphenyl exposures differentially regulate hepatic metabolism and pancreatic function: implications for nonalcoholic steatohepatitis and diabetes. Toxicol Apol Pharmacol 2019;363:22–33.
[31] Dusanov S, Ruzzin J, Kiviranta H, Klemsdal TO, Retterstøl L, Rantakokko P, et al. Associations between persistent organic pollutants and metabolic syndrome in morbidly obese individuals. Nutr Metab Cardiovasc Dis 2018;28(7):735–42.

[32] Vassilopoulou L, Psycharakis C, Petrakis D, Tsiaoussis J, Tsatsakis AM. Obesity, persistent organic pollutants and related health problems. Adv Exp Med Biol 2017;960:81–110.
[33] Jørgensen ME, Borch-Johnsen K, Bjerregaard P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance among Greenland Inuit. Diabetologia 2008;51(8):1416–22.
[34] Lee Y-M, Jacobs Jr DR, Lee D-H. Persistent organic pollutants and type 2 diabetes: a critical review of review articles. Front Endocrinol 2018;9. article 712.
[35] Henriquez-Hernandez LA, Luzardo OP, Valeron PF, Zumbado M, Serra-Majem L, Camacho M, et al. Persistent organic pollutants and risk of diabetes and obesity on healthy adults: results from a cross-sectional study in Spain. Sci Total Environ 2017:607–8.
[36] Alhassan A, Young J, Lean MEJ, Lara J. Consumption of fish and vascular risk factors: a systematic review and meta-analysis of intervention studies. Atherosclerosis 2017;266:87–94.
[37] Manninen SM, Lankinen MA, de Mello VD, Laaksonen DE, Schwab US, Erkkilä AT. Intake of fatty fish alters the size and the concentration of lipid components of HDL particles and Camelina sativa oil decreases IDL particle concentration in subjects with impaired glucose metabolism. Mol Nutr Food Res 2018;62(19): e1701042.
[38] Zibaeenezhad MJ, Ghavipisheh M, Attar A, Aslani A. Comparison of the effect of omega-3 supplements and fresh fish on lipid profile: a randomized, open-labeled trial. Nutr Diabetes 2017;7(12):1.
[39] Ruzzin J, Jacobs Jr DR. The secret story of fish: decreasing nutritional value due to pollution? Br J Nutr 2012;108:397–9.
[40] Tørris C, Småstuen MC, Molin M. Nutrients in fish and possible associations with cardiovascular disease risk factors in metabolic syndrome. Nutrients 2018;10(7).
[41] Maehre HK, Jensen IJ, Elvevoll EO, Eilertsen KE. $\omega - 3$ fatty acids and cardiovascular diseases: effects, mechanisms and dietary relevance. Int J Mol Sci 2015;16:22636–61.
[42] Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. N Engl J Med 2019;380(1):11–22.
[43] Micha R, Shulkin ML, Penalvo JL, Khatibzadeh S, Singh GM, Rao M, et al. Etiologic effects and optimal intakes of foods and nutrients for risk of cardiovascular diseases and diabetes: systematic reviews and meta-analyses from the Nutrition and Chronic Diseases Expert Group (NutriCoDE). PLoS One 2017;12(4):e0175149.
[44] Bitok E, Sabate J. Nuts and cardiovascular disease. Prog Cardiovasc Dis 2018;61(1):33–7.
[45] Rajaram S, Haddad EH, Mejia A, Sabate J. Walnuts and fatty fish influence different serum lipid fractions in normal to mildly hyperlipidemic individuals: a randomized controlled study. Am J Clin Nutr 2009;89(5):1657–63.
[46] Lee HA, Hwang HJ, Oh SY, Ha EH, Park H. Dietary patterns related to exposure to persistent organic pollutants based on the Ewha Birth and Growth Cohort. Environ Pollut 2018;243:189–96.
[47] Kahleova H, Tonstad S, Rosmus J, Fisar P, Mari A, Hill M, et al. The effect of a vegetarian versus conventional hypocaloric diet on serum concentrations of persistent organic pollutants in patients with type 2 diabetes. Nutr Metab Cardiovasc Dis 2016;5:430–8.
[48] Guo W, Huen K, Park JS, et al. Vitamin C intervention may lower the levels of persistent organic pollutants in blood of healthy women - a pilot study. Food Chem Toxicol 2016;92:197–204.
[49] Jandacek RJ, Heubi JE, Buckley DD, Khoury JC, Turner WE, Sjodin A, et al. Reduction of the body burden of PCBs and DDE by dietary intervention in a randomized trial. J Nutr Biochem 2014;4:483–8.
[50] Bou M, Berge GM, Baeverfjord G, Sigholt T, Østbye TK, Ruyter B. Low levels of very-long-chain n-3 PUFA in Atlantic salmon (Salmo salar) diet reduce fish robustness under challenging conditions in sea cages. J Nutr Sci 2017;6:e32.
[51] Li H, Zhu D, Lu X, Du H, Guan S, Chen Z. Determination and risk assessment of sixteen polycyclic aromatic hydrocarbons in vegetables. J Environ Sci Health 2018;53(2):116–23.
[52] Berntssen MHG, Julshamn K, Lundebye AK. Chemical contaminants in aquafeeds and Atlantic salmon (Salmo salar) following the use of traditional- versus alternative feed ingredients. Chemosphere 2010;78(6):637–46.
[53] Hausken T, Skaare JU, Polder A, Haugen M, Meltzer HM, Lundebye AK, et al. High consumption of farmed salmon does not disrupt the steady state of persistent organic pollutants (POP) in human plasma and adipose tissue. J Toxicol Environ Health 2014; 77(20):1229–50.
[54] Myrmel LS, Fjære E, Midtbø LK, Bernhard A, Pettersen RK, Sonne SB, et al. Macronutrient composition determines accumulation of persistent organic pollutants from dietary exposure in adipose tissue of mice. J Nutr Biochem 2016;27:307–16.
[55] https://sjomatdata.hi.no/#seafood.