Short-term dietary interventions in persons with cardiovascular risk factors

PhD thesis

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None of these studies included physical activity, which has been studied in earlier trials originating from this section. With this exception, the current thesis reflects an essential part of our daily clinical work: lifestyle-intervention in persons at increased risk for cardiovascular disease due to cardiometabolic risk factors as hypercholesterolemia and other lipid disturbances, metabolic syndrome, obesity and smoking. I am grateful to all our study participants, without their interest the trials would not have been possible.

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Abbreviations

ANOVA	analysis of variance
BMI	body mass index
CAD	coronary artery disease
CHD	coronary heart disease
CRP	C-reactive protein
CVD	cardiovascular disease
DASH	Dietary Approach to Stop Hypertension
GI	glycemic index
GL	glycemic load
HDL	high density lipoprotein
HOMA-IR	homeostasis model assessment of insulin resistance
ICAM	intracellular adhesion molecule
IL	interleukin
LDL	low density lipoprotein
MNWS	Minnesota Nicotine Withdrawal Symptoms
MCP	monocyte chemoattractant protein
MI	myocardial infarction
NCEP ATP	National Cholesterol Education Program, Adult Treatment Panel
NRT	nicotine replacement therapy
PAI	plasminogen activator inhibitor
PCSK9	proprotein convertase subtilisin/kexin type 9
PREDIMED	Prevención con Dieta Mediterránea
RCT	randomized controlled clinical trial
RMR	resting metabolic rate
TNF	tumor necrosis factor
TQD	target quit date
VCAM	vascular adhesion molecule
WHI	Women Health Initiative
WHO	World Health Organization

List of papers

Paper I

Plant sterols from rapeseed and tall oils: Effects on lipids, fat-soluble vitamins and plant sterol concentrations
Heggen E, Granlund L, Pedersen JI, Holme I, Ceglarek U, Thiery J, Kirkhus B, Tonstad S.
Nutr Metab Cardiovasc Dis 2010; 20: 258-65.

Paper II

Effects of margarine enriched with plant sterol esters from rapeseed and tall oils on concentrations of markers of endothelial function, inflammation and hemostasis.
Heggen E, Kirkhus B, Pedersen JI. Tonstad S.
Scand J Clin Lab 2015; 75(2): 189-92.

Paper III

Effect of a low fat versus a low glycemic load diet on inflammatory biomarker and adipokine concentrations
Heggen E, Klemsdal TO, Haugen F, Holme I, Tonstad S.
Metab Syndr Relat Dis 2012; 10: 437-42.

Paper IV

Low carbohydrate and moderately fat-reduced diets similarly affected early weight gain in varenicline-treated overweight or obese smokers
Heggen E, Svendsen M, Klemsdal TO, Tonstad S.
Nicotine Tob Res 2016; 18(6): 1440-8.

Paper V
Smoking cessation improves cardiometabolic risk in overweight and obese subjects treated with varenicline and dietary counseling.
Heggen E, Svendsen M, Tonstad S.
Submitted, Nutr Metab Cardiovasc Dis September 2016.

1 General introduction

1.1 Scope of cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of deaths globally as in Norway [1, 2]. Death rates from CVD reached the highest point in Norway in the early 1970s and have since decreased dramatically. This decline has continued into the present century, and since the year 2000 to present, death rates have been almost halved [2] (Figure 1). This trend has also been observed in many Western countries. These improvements have been attributed equally to changes in risk factors as to improved treatments [3].

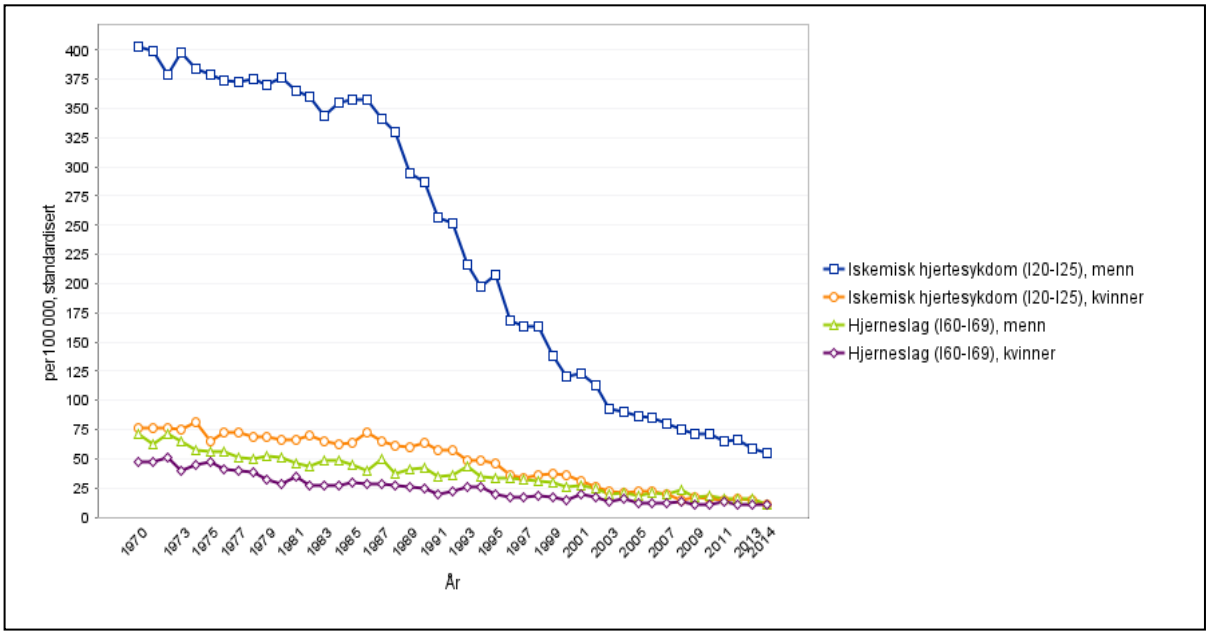

Figure 1. Mortality from ischemic heart disease and stroke for men and women 45-64 years in Norway 1970 - 2014.
Data from the Norwegian Institute of Public Health.

Despite these encouraging changes, large disparities in CVD mortality remain, due to region, social class or educational level – also in Norway [4]. Furthermore, due to better treatments and the aging of the population it is expected that more people will be living with CVD in future years [2]. Thus, CVD is likely to remain a major cause of premature mortality, even in countries experiencing improvements in death rates. One of the postulated causes is the obesity epidemic. According to the World Health Organization (WHO) the prevalence of

obesity more than doubled world-wide between 1980 and 2014. In 2014, 39% of adults were overweight (body mass index [BMI] 25-30 kg/m^2) and 13% were obese (BMI ≥ 30 kg/m^2) [5]. In Norway, the HUNT study showed that more than 20% of men and women aged 30-59 was obese in 2006-2008 [6]. A major consequence of the rising tide of obesity is an expected increase in the incidence of type 2 diabetes. Participants in the HUNT study whose BMI was ≥ 30 kg/m^2 had 20 times the risk of type 2 diabetes during 11 years of follow-up as those with BMI of 22 kg/m^2 [7]. These data further demonstrated an increase in the prevalence of type 2 diabetes among adults from 2.9% of the population to 4.3% between 1984 and 2008 [8]. Much higher numbers have been reported from Oslo where a prevalence of 20% has been shown among specific ethnic groups including those of Pakistani and Sri Lankan origins [9].

1.2 Risk factors for cardiovascular disease

To predict and prevent CVD we must recognize and if possible treat the causal risk factors. Risk factors may be causal or just a marker of risk - often called "innocent bystanders" [10]. The WHO has ranged high blood pressure, tobacco use, physical inactivity, unhealthy diet, high blood glucose, high cholesterol, overweight and obesity as the main causal factors of the global burden of disease [1]. Traditionally, risk factors have been classified into preventable and non-modifiable factors as age, gender and ethnicity. In the INTERHEART case-control study of incident acute myocardial (MI) in 52 countries it was found that nine modifiable risk factors (apolipoproteins, smoking, hypertension, diabetes, abdominal obesity, psychosocial stress, physical activity, alcohol and diet) were associated with acute MI in both men and women and explained more than 90% of the population attributable risk [11].

The major study designs that have been used to establish causality include prospective observational studies and randomized controlled clinical trials (RCTs). RCTs have demonstrated that lowering of LDL-cholesterol and elevated blood pressure lower the risk of CVD. On the other hand, neither antioxidant supplementation, vitamins to lower homocysteine nor estrogen therapy confirmed the salutary effects of these interventions as seen in observational studies [12, 13, 14].

Conducting large scale RCTs with adequate power to detect reductions in clinical endpoints is extremely expensive, takes years, requires very large sample sizes and is nowadays almost solely possible in the context of drug trials sponsored by the pharmaceutical industry. In recent years a new study design has been developed, the Mendelian

randomization studies. These studies attempt to distinguish causal factors from mere markers of disease. These studies are parallel to RCTs as they investigate the effect of nature's own randomization process on the risk of disease.

LDL-cholesterol and other lipids

The Cholesterol Treatment Trialists' Collaboration consists of a consortium of researchers who *a priori* planned how to conduct meta-analysis of the effects and safety of LDL-lowering on CVD endpoints. In a recent meta-analysis among 174 000 participants in 27 randomized trials, each 1 mmol/l of reduction in LDL-cholesterol led to a 16% reduction in major vascular events in women, and a 22% reduction in men. These benefits translated into all-cause mortality reductions of about 10% in men and women [15]. Lowering of LDL-cholesterol reduces CVD independently of baseline levels [16]. Given the robustness of data linking LDL-cholesterol to CVD, the question may be asked whether LDL-cholesterol is an adequate surrogate endpoint of CVD. However, interventions that lower LDL-cholesterol may have undesirable pleiotropic or accompanying effects that may increase risk of CVD as has been shown related to estrogen replacement therapy [14] and cholesterol-ester transfer protein inhibitors [17].

Trials of drugs modifying other lipid fractions including HDL-cholesterol and triglycerides have not shown consistent benefits on CVD [17, 18]. The subclass or biological activity of HDL may be of more importance than the concentration [10]. Mendelian randomization studies have questioned the causality of HDL and triglycerides [19]. Lately, evidence indicates that remnant cholesterol may be causally related to CHD [20].

Apolipoproteins constitute the protein part of lipoproteins. Atherogenic lipid particles contain apolipoprotein B while HDL contains apolipoprotein A1. The apolipoprotein B/A1 ratio is one of the strongest risk markers of CVD [11]. Because of lack of availability and costs of adding these measurements to routine lipid profiles, their use is not generally recommended [21].

Blood pressure

High blood pressure is a major risk factor for CVD. Mortality rates from coronary artery disease (CAD) and cerebrovascular disease increase progressively from low normal blood pressure levels [21]. Based on individual patient data from 11 trials including over 67 000 individuals, meta-analysis showed that lowering blood pressure provides similar relative

protection at all levels of baseline risk. [22]. As with lowering of LDL-cholesterol, absolute risk reduction increases as baseline risk increases.

Cigarette smoking

Cigarette smoking is a classical and major risk factor in the development of CVD and atherosclerosis as well as a promoter of thrombosis. The 10 year risk of fatal CVD is doubled in smokers and the relative risk in smokers <50 years old is 5 times as high as in non-smokers [21, 23]. Data from Norway has led to the conclusion that smoking 1-4 cigarettes per day is associated with a significantly higher risk of dying from ischemic heart disease and from all causes [24]. WHO states that "there is no safe level of exposure to tobacco smoke" [25]. Mendelian randomization studies have supported a causal association between smoking heaviness and both resting heart rate [26] and a relative increase in waist circumference [27].

Overweight and obesity

Observational studies demonstrate a relation between obesity and mortality. However, the dose-relationship has been questioned, as not all studies show that overweight (BMI 25-30) increases mortality or may even decrease risk compared to normal weight [28]. Recently, meta-analysis that included 230 cohort studies among over 30 million participants showed that both overweight and obesity were associated with increased risk of all-cause mortality and the nadir of the curve was observed at BMIs of 23-24 kg/m^2 among never smokers, 22-23 kg/m^2 among healthy never smokers, and 20-22 kg/m^2 with longer durations of follow-up. [29]. To date, RCTs demonstrating that treatment of obesity lower CVD mortality are not available, both due the expense of such trials, and concurrent other treatments for CVD risk factors that may normalize risks associated with obesity. When CVD risk factors are adequately controlled, the risk associated with obesity tends to diminish [30]. Furthermore, weight loss involves both loss of adipose tissue and muscle mass, and may be disadvantageous in some conditions [31].

Metabolic syndrome and insulin resistance

Metabolic syndrome consists of a constellation of abdominal obesity, hypertension, dyslipidemia and dysglycemia contributing to CVD and type 2 diabetes directly and by creating a pro-inflammatory milieu [32]. Metabolic syndrome doubles the risk of CVD; however, whether the strength of the association is greater than the effect of each single factor is debated [33]. WHO, expert panel groups and organizations have used different definitions

of metabolic syndrome, but they agree on the components involved [34, 35]. Table 1 show the criteria used in the studies of the present thesis.

Table 1. Definitions of metabolic syndrome.

Component (cardiometabolic risk factor)	**NCEP ATP III criteria 2001** ≥3 of	**Harmonizing criteria 2009** ≥3 of
Abdominal obesity (waist circumference)		
Men	≥102 cm	≥102 cm*
Women	≥88 cm	≥88 cm*
Triglycerides	≥1.7 mmol/L	≥1.7 mmol/L
HDL-cholesterol		
Men	<1.0 mmol/L	<1.0 mmol/L
Women	<1.3 mmol/L	<1.3 mmol/L
Blood pressure	≥130/≥85 mmHg or antihypertensive medication	≥130/≥85 mmHg or antihypertensive medication
Fasting glucose	≥6.1 mmol/L or antidiabetic medication	≥5.6 mmol/L or antidiabetic medication

*Recommended threshold for European populations.

There is a close correlation between components of metabolic syndrome and insulin resistance. While the gold standard for establishing the presence of insulin resistance is the hyperinsulinemic-euglycemic clamp method, this is an invasive and time consuming procedure. The Homeostasis Model Assessment of insulin resistance (HOMA-IR) is a model

or equation using basal glucose and insulin concentrations and is considered to be a robust tool for the surrogate assessment of insulin resistance.

Inflammation

Inflammation is an important part of the atherosclerotic process [36]. The presence of subclinical inflammation is suggested by an elevated concentration of high sensitivity C-reactive protein (CRP) and increased concentrations of other inflammatory markers. Epidemiological studies suggest strong association between high-sensitivity CRP concentrations and CVD risk [37]. On the other hand Mendelian randomization studies did not identify associations between CRP coding gene-variants and CVD [38, 39].

A host of other markers of inflammation as the cytokines have been studied including interleukins which are signal molecules produced by a wide variety of cells, also in adipose tissue. Some cytokines promote inflammation while others are protective. In normal intima of the arterial wall T-cells and macrophages may express the pro-inflammatory interleukin-1 and interleukin-6 (IL-1 and IL-6) and tumor necrosis factor α (TNFα). Variation in the IL-6 receptor coding gene was associated with CVD [38, 39] supporting the notion that inflammation plays a causal role in atherosclerosis. Cholesterol microcrystals are one of the best known triggers of this inflammatory process. The chemokine monocyte chemoattractant protein 1 (MCP1) and its pathways are shown to have a central role in the initiation of atherosclerosis recruiting circulating monocytes that bind to the endothelial adhesion molecules like E-selectin, vascular or intra-cellular adhesion molecules (VCAM and ICAM). Monocytes differentiate to macrophages, migrate through the endothelium, and phagocyte oxidized LDL turning into foam-cells [39]. In genetic studies E-selectin polymorphisms have been related to hypertension [40]. Large scale studies have been started to assess the effect of low dose methotrexate and canacinumab, a monoclonal antibody binding IL-1β, on CVD [39].

Adipokines

Adipokines or adipose-derived hormones are cell signaling proteins secreted by adipose tissue. Adipokines play a role in a variety of pathophysiologic mechanisms related to atherosclerosis, including the regulation of lipid and glucose metabolism, endothelial function, blood pressure and coagulation. Leptin was the first so-called adipokine to be discovered in 1994. Since then many others have been identified, some of which are associated with inflammation and CVD, while others appear to be protective. Leptin has a

regulatory effect on appetite as well as pro-inflammatory effects, while adiponectin has anti-inflammatory effects through up and down-regulation of different cytokines. Resistin in humans is mostly produced by macrophages and monocyte induced by other cytokines and its role in inflammation is not unambigously, mostly pro-inflammatory. Obese individuals have increased serum concentrations of leptin and resistin and lower adiponectin levels compared to lean [41]. Adipokines and cytokines may have different functions in normal-weight and obese individuals, in different tissues and in acute or chronic conditions [42].

Thrombosis

Plasminogen activator inhibitor 1 (PAI-1) is an inhibitor of fibrinolysis, is pro-trombotic and pro-inflammatory. Its expression is mediated by cytokines including IL-6 and TNFα [41, 43]. Serum concentrations are increased in obese persons [44] and lowered by statin treatment [44]. In observational studies PAI-1 seems to be associated with type 2 diabetes [45].

1.3 Reducing cardiovascular risk

Health benefits from preventive interventions may not be recognized in the short term. Recommended lifestyle changes may require longtime personal efforts often before any symptoms have appeared and sometimes with no measurable effects. Population based strategies and incentives are important to facilitate preventive interventions [46].

Lifestyle

A healthy lifestyle remains the cornerstone in prevention of CVD and acts on several CVD risk factors and diseases. Lifestyle modifications may give clinical important benefits in subjects not fulfilling the criteria for pharmacological treatment and are especially important considering lifetime risk. Cardiovascular disease prevention guidelines recommend lifestyle and dietary changes as a key component in lipid-lowering therapy [21]. Among persons treated with medications, lifestyle improvements may help to reduce the dosage and minimize side effects and costs. According to a cross-sectional study from 1999- 2010 statin users had a higher increase in intake of calories and fat and in BMI than subjects not on statin [47]. These findings remind us of the importance of reinforcing the dietary recommendations along with medication. A healthier lifestyle pattern implies health effects beyond lipids; including

physical fitness, body weight, blood pressure, glycemic parameters, sleep disturbances, psychological conditions and a wide spectrum of disease.

Lifestyle may be due to genetic predisposition or acquired behavioral patterns sometimes from childhood and the changes are difficult to implement and adhere to. Recommendations from media as well as from health care givers may be confusing. Strategies based on cognitive behavioral therapy and motivational interviewing techniques are encouraged. The following elements in counseling are emphasized: Use enough time to make a relationship and explore the patient's motivation and barriers, give recommendations in an understandable language, acknowledge personal "change talk", experiences and ideas to ensure commitment, help setting realistic goals, agree on self-monitoring and offer support and follow-up [21, 48].

Stopping smoking

There is no other intervention as effective as quitting smoking for improving health and longevity [23]. The benefits of quitting occur almost immediately. The excess risk of CHD caused by smoking is almost halved after 1-2 years of smoking cessation, after controlling for relevant confounders [23, 49]. However even with optimal assistance quit rates are still low. Nicotine replacement therapy (NRT), bupropion and varenicline are all medications that increase the rates of quitting. Varenicline is the most effective medication [23, 50, 51].

Weight gain when stopping smoking seems to be almost inevitable, but does not offset the cardiovascular benefits of quitting [52]. However, some quitters gain a lot of weight and the risk of diabetes is temporarily increased [53, 54]. Dieting to avoid or reduce weight gain when stopping smoking is of special interest because nicotine and food share the same rewarding pathways in the brain [55]. A restricted diet could give more craving, lower quit rates and more relapses [56, 57]. A Cochrane review found insufficient data to make strong clinical recommendations for effective programs to prevent weight gain after cessation [58]. Personalized weight management support and exercise may reduce weight gain after one year without impairing quit rates [58, 59].

Physical activity

Increased levels of physical activity are beneficial to cardiovascular and general health. Guidelines include strong recommendations about physical activity [21]. Recent reviews summarize the effects of different types and volumes of exercise in prevention and treatment of CVD and emphasize the importance of promoting exercise training in our patients as in the

whole population [60, 61]. None of the studies in the present thesis included interventions to increase physical activity.

Multiple interventions

Studies evaluating the effects of multiple risk factor interventions are conflicting. A recent meta-analysis concludes that "When it comes to lifestyle recommendations, more is sometimes less" [62]. There was a curvilinear association between number of recommendations for behavioral changes in diet, smoking and physical activity and measured effects. A moderate number of interventions appear to give the greatest change [62].

A review of 74 trials conclude that intensive lifestyle (diet and physical activity) counseling in persons with risk factors for CVD improved intermediate health outcomes for 12-24 months. There was no reduction in CVD events or mortality in the few trials reporting these outcomes. [63]. However, 40-years follow up of the Oslo diet and antismoking study giving lifestyle advices for 5 years, reported long-term reduced risk of CHD mortality [64].

Large intervention studies for diabetes prevention in Europe and US combined dietary advices and exercise in subjects at risk for diabetes and have confirmed significant reduction in incidence of diabetes for up to 15 years [65, 66].

1.3 Dietary intervention

Randomized controlled trials of CVD

In contrast to drug intervention studies, studies of dietary interventions require persons to change lifestyles and habits that may be entranced for many years. Such studies are costly, requiring the investment of time and resources to follow-up participants. The emergence of differences in CVD may take years, if not decades. Despite these barriers, a small handful of trials have been conducted that have examined incidence of CVD endpoints between various dietary interventions:

The Lyon Diet Heart Study [67] starting in 1988 showed sustained protective effect of a Mediterranean type diet for 4 years in secondary prevention of CHD. The Women Health Initiative (WHI) Randomized Controlled Dietary Modification-study evaluated a fat-reduced diet with increased intake of fruit, vegetables and grains, without any significant reduction in the risk of CHD, CVD or stroke in nearly 50000 postmenopausal women over 8 years follow-up [68]. The PREDIMED (Prevención con Dieta Mediterránea) trial included about 7500 men

and women with type 2 diabetes or at least 3 other risk factors randomized to a Mediterranean diet supplemented with either olive oil or nuts or a control fat-restricted diet. After a median follow up of 4.8 years the two Mediterranean diets showed a protective effect on the composite end point of myocardial infarction, stroke and death from CVD, significant only for stroke alone [69].

The Look AHEAD study evaluated intensive lifestyle intervention in patients with type 2 diabetes with or without CVD. The intervention group was intended to reduce weight by increased physical activity and a diet restricted in calories and fat and maintained a modest weight reduction. However the trial was terminated prematurely after 9-10 years because there was no significant difference between the intervention and control group in CVD events [70].

In conclusion these studies suggest that a Mediterranean type diet, where energy from foods rich in saturated fat and sugars is substituted with energy from olive oil or nuts may prevent CVD [71]. In contrast, low fat diets do not seem effective in preventing CVD.

Studies to reduce high cholesterol concentrations

Numerous observation and interventional nutritional studies followed by systematic reviews and meta-analyses have been conducted to understand the effects of reduced saturated fat intake on blood lipids and thereby on risk of CVD. Currently, the thinking is that saturated fat should be replaced by dietary unsaturated fat, as suggested in meta-analysis [72, 73]. Replacement by certain carbohydrates is likely as deleterious as saturated fat. An updated Cochrane review of 44 randomized trials with at least three months duration found that dietary advice reduced total cholesterol by 0.15 mmol/l and LDL-cholesterol by 0.16 mmol/l while HDL-cholesterol and triglycerides were unchanged [74]. Furthermore it was concluded that dietary advice appears to be effective over about 12 months, but longer-term effects are not known.

Nutraceuticals for the treatment of high cholesterol concentrations

The word nutraceuticals is made up from the words nutrition and pharmaceutical and is defined as a food or a part of a food giving health benefit, including supplements and functional food. [75]. A recent review concluded that plant sterols/stanols and red yeast rice are nutraceuticals that have significant cholesterol-lowering effects [75].

Plant sterols and the saturated stanols similarly reduce total and LDL-cholesterol [76] and some studies report a triglyceride lowering effect [77]. When given on the top of statin or

ezetemibe therapy, plant sterols/stanols have an additive effect on LDL-reduction [77]. Intake of food enriched with plant sterols increases plasma concentrations of plant sterols and could be of concern due to their possible atherogeneticy. Mendelian randomization studies have indicated an association between plant sterol raising gene variants and CAD, but the causality is questioned as these variants also give higher cholesterol concentrations [77]. There are many clinical trials and population based studies in this field, but so far no hard-endpoint studies. However, based on the LDL-cholesterol lowering effect and the absence of adverse signals, the European Atherosclerosis Society Consensus Panel in 2014 recommended intake of plant sterols/stanols to be considered combined with other lifestyle interventions to achieve LDL targets, alone or in addition to pharmacotherapy [77]. Functional food with plant sterols or plant stanols is approved by the European Food Safety Authority (ESFA) and Food and Drugs Administration [75]. A dose of 2 g/day is recommended by the 2016 European guidelines in cardiovascular disease prevention [21].

Studies to promote weight reduction

Classical dietary interventions for weight control have focused on energy restriction as the primary method of promoting weight reduction (in addition to physical activity and behavior modification). Dietary based weight loss should preferably also improve risk factors for metabolic syndrome and CVD. In terms of reducing lipids and CVD risk, controversy exists as to the optimal macronutrient composition. Diets low in fat promote weight loss regardless of attempts to lose weight [78] and restriction of saturated fat has been generally recommended because of the reduction of LDL-cholesterol. However, in patients who are insulin resistant in addition to being overweight or obese, there may be a lesser responsiveness to the cholesterol-lowering effects of limiting saturated fat [79]. Furthermore, lowering fat often leads to increasing dietary carbohydrates. Of greatest concern are carbohydrates with a high glycemic index, indicating the total rise in a person's blood sugar level following consumption of the food.

Glycemic index (GI):
the blood glucose raising potential of the carbohydrate content of a food, usually 50 g compared to 50 g pure glucose (GI 100).
High GI ≥70, intermediate 56-69, low-GI ≤55.

Glycemic load (GL):
is calculated by multiplying the GI by the amount of carbohydrate in grams provided by a food serving and then dividing the total by 100.
High GL≥20, intermediate11-19, and low ≤10.

Increased carbohydrates can worsen the dyslipidemia component of metabolic syndrome (so-called "atherogenic dyslipidemia"). Concern has been expressed that dietary recommendations focusing on an upper threshold for saturated fat may not be suitable for overweight/obese persons with metabolic syndrome [80].

Intense interest has focused on alternative diets to low fat ones. Diets rich in protein and in carbohydrates that are low in glycemic index are associated with improved satiety and appear to be effective weight control treatments [81]. In trials with high compliance to each group (fat percentage differing by more than 5% of energy), high fat diets appear to lead to greater weight loss than low-fat ones [82]. Meta-analysis that included only trials with strict low-carbohydrate limits showed increases in LDL-cholesterol levels compared to low-fat diets, but this meta-analysis included so called "healthy" overweight persons, that did not have features of insulin resistance [83]. Another meta-analysis in overweight and obese including metabolic risk factors, looked at the predicted risk of CVD using a pooled cohort equation and found a modest, but statistical significant greater reduction in predicted risk in the low-carbohydrate compared to the low-fat diets [84].

An important consideration in choice of dietary pattern for weight loss is patient preferences and adherence. It has been demonstrated that adherence to any dietary program (behavioral adherence) determines weight loss to a greater extent than the type of diet [85]. High fat diets may be associated with better dietary adherence [86], though this may not be the case in all settings. Surprisingly, a trial allowing personal choice of one of two diets even with allowance to switch diet, found no improvement in weight loss compared to randomly assignment to the same diets [87]. Individual biological effects of diets, personal preferences and adherence could all be influenced by genetic variations [81, 88].

Studies of diet and inflammation

Weight loss reduces inflammatory biomarkers. Furthermore, epidemiological studies have documented a link between dietary patterns and markers of inflammation, however the role of different diets is not fully established [89]. A review of studies using an index of dietary inflammation has shown an association between the score and risk of CVD, metabolic syndrome and overall mortality [90].

A systematic review and meta-analysis found a beneficial effect on CRP of low vs high glycemic index/load diets [91]. A review of RCTs providing all food and beverage in diets differing in GI and GL concluded with weak or no effect on inflammatory markers, one study showed lower IL-6 and TNFα in the low GI diet group [92]

A recent systematic meta-analysis of randomized trials with a Mediterranean diet concluded that this dietary pattern improved CRP, IL-6 and adiponectin, as well as ICAM and E-selectin; some of the results were based on few studies [93]. In a cross-sectional study resistin showed positive association to saturated fat, negative to monounsaturated fat intake and with adherence to a Mediterranean diet [94].

Studies of diet and blood pressure

Lifestyle intervention is recommended for all persons having suboptimal blood pressure, white-coat hypertension or grade 1 hypertension with low total risk. Lifestyle changes can help reduce dosages of antihypertensive medication and improve achievement of treatment goals. Exercise, weight reduction, and reduction of dietary sodium are among the most important changes [21, 95]. A systematic review and meta-analysis included studies with more than 23000 participants [96]. The overall pooled effect of diet was a 3.1 mmHg reduction in systolic and 1.8 mmHg reduction in diastolic blood pressure. The best effect was seen in the DASH and other low-sodium studies.

1.4 Summary

Research conducted in the last two decades has resulted in improved knowledge in the field of nutrition and CVD, but several questions remain. Individual or personalized diets based on genetics and other differences in biological and psychological effects have been given a lot of interest. The gastrointestinal microbiome and microbial balance are shown to be of importance for human metabolism and can be altered by diet [97]. However further research is needed before microbial or gene-diet interactions will have implications for clinical practice and these topics are not a part of this thesis.

A recent review highlighted the importance of evaluating the diversity of cardiometabolic risk factors - not only lipids or obesity [98]. Focusing on food habits and dietary patterns, rather than single isolated nutrients was emphasized because of the complex and sometimes synergistic effects both on cardiometabolic risk, weight and general health. In the current thesis, we examined effects of diet on the classical risk markers (lipids and obesity) as well as on risk factors as metabolic syndrome and markers of inflammation. Furthermore, the dietary interventions focused on foods, nutraceuticals and dietary patterns rather than single nutrients. Energy-restricted diets for weight reduction were chosen to be protective towards CVD, according to knowledge at the time that the studies were planned.

2 Study aims

The overall aim of this thesis was to examine the effect of different dietary interventions on a broad spectrum of cardiovascular risk factors in individuals at increased risk of cardiovascular disease.

Papers I and II (Study 1)

To assess and compare the effect of two margarines with different plant sterol profiles to a margarine with no added plant sterols on lipids, fat-soluble vitamins, serum plant sterol concentrations and markers of endothelial function, inflammation and hemostasis.

Paper III (Study 2)

To examine and compare the effect of two mildly hypocaloric diets, one low in fat and one low glycemic load diet - on inflammatory markers and adipokines in overweight and obese men and women with one or more criteria of the metabolic syndrome.

Papers IV and V (Study 3)

To compare the effect of a low carbohydrate versus a moderately fat-reduced diet on weight gain in overweight and obese smokers trying to quit, and examine the effect of smoking cessation on cardiometabolic risk factors.

3 Ethics

The Regional Committees for Medical and Health Research Ethics in Norway evaluated the studies and the work was conducted in accordance with the Declaration of Helsinki. Oral and written informed consent was obtained by the study physician before any study-procedures were performed.

4 Research design and methods

4.1 Participants and study design

The Preventive Cardiology section of the Department of Endocrinology, Morbid obesity and Preventive Medicine at Oslo University Hospital is a referral center for primary and secondary prevention of CVD. Referrals originate from primary care physicians and other hospital departments. This thesis is based on three randomized clinical trials performed at the clinic.

After informed consent procedures and screening the inclusion and exclusion criteria were evaluated and eligible subjects were randomized according to a computer-generated list prepared by a statistician. Only study 1 was blinded, matching "placebo" margarine was provided and participants and study staff were blinded. Study 2 and 3 examined different diets and blinding was not possible. Key features of the studies are summarized in table 2.

Table 2. Inclusion criteria and study designs

Paper	Intervention	Study design	Population	No of participants	Outcome	Duration
I &II	Study 1. Plant sterols	Randomized, double blind, crossover	Men and women with hypercholesterolemia defined as total cholesterol 5.0–7.5 mmol/L	61	Lipids, fat-soluble vitamins, inflammatory markers	4 weeks x 3 with 1 week wash-out in between
III	Study 2. Low glycemic load diet	Randomized, parallel groups	Overweight and obese persons with BMI 28-35/40 kg/m^2 and ≥ 1 metabolic syndrome component	181	Body weight, inflammatory markers, adipokines	3 months with 12-month final follow-up
IV&V	Study 3. Comparison of two weight loss diets parallel to stopping smoking	Randomized, parallel groups	Overweight and obese persons who smoked ≥10 cigarettes/day BMI 25-40 kg/m^2	122	Body weight, components of metabolic syndrome	6 months

Participants in all three trials were men and women with a mean age of 50 years. They were recruited among patients treated at the clinic and by newspaper advertisement (Table 3)

Table 3. Participant characteristics

	Study 1	**Study2**	**Study 3**
Randomized (No)	61	202	122
Drop-outs before 3 months (No)	2	12	14
Completing study %	97	81	78
Mean age (years)	52± 12	50± 9	50± 9
Women (%)	28	58	73
Baseline BMI (kg/m²)	24.8 ± 2.9	33.2 ± 2.9	30.5± 3.6
Serious adverse event			
Death	1		
Cerebrovascular event	1		

Study 1 had a double blinded, cross-over design. Eligible participants were men and women aged 25–75 years with moderately elevated cholesterol levels (total cholesterol 5.0–7.5 mmol/L) and BMI < 29 kg/m^2. Exclusion criteria were triglycerides > 4.0 mmol/L, established CVD, an indication for statin therapy, uncontrolled hypertension, secondary hyperlipidemia, diabetes and medications affecting lipids including postmenopausal hormones. Chronic rheumatic disease, pregnancy and breastfeeding were additional exclusions. The participants were randomized to use the three margarines in one of six possible sequences with one-week washout in-between.

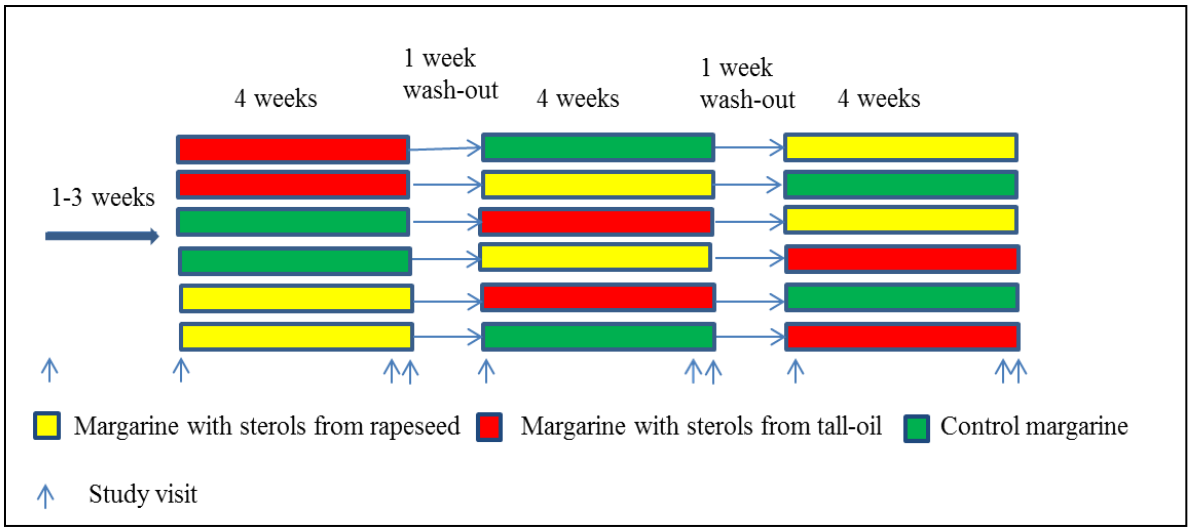

Figure 2. Design of study 1

Study 2 had a randomized parallel groups design. Inclusion criteria were men and women aged 30–65 with BMI 28-40 kg /m2 for men, and 28-35 kg /m2 for women and one or more metabolic syndrome criteria. Exclusions were symptomatic cardiovascular disease, lipid-lowering and antidiabetic medication as well as medication for weight reduction. Subjects were randomized to follow one of two diets.

Study 3 also had a randomized parallel groups design. Randomization was done in blocks of 8. Participants were overweight or obese (BMI 25-40 kg/m^2) men and women aged 20-65 years, who smoked ≥10 cigarettes daily, were motivated to quit and willing to be treated with varenicline to aid cessation. Exclusion criteria were the occurrence of a cardiovascular event within 2 months prior to screening, heart failure NYHA class III-IV, diabetes mellitus type 1 or type 2 treated with insulin, history of serious psychiatric disorder, ongoing major depression or anti-depressive medication, alcohol or drug abuse, pregnancy and lactation. Other exclusion criteria were history of bariatric surgery, use of drugs for weight loss or participation in a weight loss program within the last four weeks, recent change in weight (>4 kg during the last 3 months), vegetarian diet, and gastrointestinal or other disorder impairing compliance with dietary recommendations. Following screening subjects returned 1 week later for randomization to 1 of 2 diets. Treatment with a 12-week course of varenicline was started 4 days thereafter. The target quit date (TQD) was 10 days after the initiation of varenicline. Follow-up visits were scheduled weekly after baseline to the 4-week post-TQD

visit and thereafter biweekly to the 12-week post-TQD visit. At each study visit, the study physician or trained nurses provided motivational counseling for cessation.

4.2 Methods

Study 1

The test margarines had added plant sterols from rapeseed or tall oil and the control had no added plant sterols. All margarines were fat-reduced and had a similar fatty acid composition. The margarines were indistinguishable in appearance and packed in color-coded boxes. The study staff and subjects were blinded to the type of margarine. The assigned dose of margarine was 25 g/day containing 2 g/day of plant sterols.

At randomization and at the start of each subsequent 4-week period the dietitian demonstrated the correct amount of margarine and how to use the margarine. Compliance was evaluated according to left-over margarine in returned boxes. Fasting blood tests for lipids were taken twice in the last week of each period, other tests as vitamins, sterols and inflammatory parameters were taken once at the end of each period.

Study 2

The interventional diet was one of two mildly hypocaloric diets, a low-fat diet (<30% fat), or a low-glycemic-load diet (30%–35% carbohydrates) as shown in table 4. The main study had 12 months follow up and dietitians met with participants at 9 regularly scheduled clinic visits. Weight and blood pressure were.measured with standard methods. At 3 months a 7-day dietary record was obtained, coded by the Institute of Nutrition Research at the University of Oslo. Blood tests for inflammatory biomarkers and adipokines were taken at randomization and after 3 months.

Study 3

Randomization diets were equally calorie-restricted, either a low-carbohydrate diet planned to provide ≤20 percentage energy (E%) from carbohydrates and ≥25 E% from protein, or a moderately fat-reduced diet planned to provide ≤30 E% from fat and ≤20 E% from protein (table 4).

At each visit participants reported the number of cigarettes smoked since the last visit. Exhaled carbon monoxide (CO) concentrations in parts per million (ppm) were tested using a Bedfont piCO+ Smokerlyzer at each visit. The Fagerström Test for Nicotine Dependence was

administered at screening. The Minnesota Nicotine Withdrawal Symptoms (MNWS) questionnaire was administered at the TQD, and post-TQD visits.

Body weight was measured on a digital and calibrated scale (Seca 770) in light indoor clothing without shoes. Waist circumference was measured midway between the lowest rib and iliac crest. Blood pressure was measured three times at 2-minute intervals after the participant rested quietly in a sitting position for at least 5 minutes.

Resting metabolic rate (RMR) was measured at the baseline and 4-week post-TQD visits using the ventilated-hood system Vmax Spectra 229 indirect calorimeter (SensorMedics). The subjects fasted overnight and refrained from smoking and heavy physical activity in the morning before the test was performed according to standardized procedures.

Participants completed a weighed dietary record during 7 days before randomization and before the visit 4-week post TQD. Energy intake was calculated using software "Mat på data" 5.0 based on the Norwegian food composition table. Physical activity was measured for 7 consecutive days as the participant wore an Actigraph GT3X+ accelerometer (ActiGraph, Fort Walton Beach, FL) at the same time points. The Actigraph software ActiLife v6.11.4 was used to calculate physical activity energy expenditure. Fasting blood samples were taken at randomization, 4 weeks and 12 weeks post TQD.

4.3 Dietary interventions

Registered dietitians and trained nutritionists counseled participants on the required changes in all the studies. The writer of this thesis and other physicians helped to explain the diet and its importance and promoted adherence.

In study 2 the dietary advices were given individually and in group sessions four times during the first three months' period (at randomization, week 2, week 4 and week 8). All participants were prescribed a moderate energy reduction of -500 kcal/day based on estimated energy requirements. The advices were individualized according to each diet and recipes were provided. Participants in the low-carbohydrate/low glycemic load group were counseled to reduce their portions of bread, potatoes, rice and pasta and to choose the low glycemic variants such as pumpernickel bread and whole meal pasta. Further they were asked to include protein rich food items in every meal. Carbohydrate containing drinks, snacks and desserts were to be avoided as much as possible. Nuts were recommended as snacks. The fat-reduced group was asked to eat low fat dairy products, lean meats and smaller portions of oil-

containing food and to increase the intake of fruit and vegetables as well as other fiber-rich carbohydrates. They were to avoid fatty snacks and desserts as much as possible, fruit and vegetables were recommended as snacks.

In study 3 dietary advice and support were given at each study visit. Both diets were equally reduced in energy by 500 kcal/day. Energy requirements were estimated based on measured RMR and level of physical activity. Individual meal plans were made with written dietary information, substitution lists, recipes and tips for planning meals. Participants in the low-carbohydrate group were recommended to reduce intake of bread, pasta, rice and all sugar-containing foods and drinks and to increase intake of oils, dressings, mayonnaise, soft margarine, nuts, meat, chicken, fish, shellfish and cheese. The moderately fat-reduced diet group was counseled to reduce intake of oils, dressings, margarine, mayonnaise, nuts, chips, desserts and chocolate and to increase intake of bread, rice, pasta, fruit, vegetables and low-fat dairy products.. For the first 7 days after TQD a daily lunch and a snack were provided as an example of each assigned diet.

Table. 4. Planned and actual macronutrients and energy intake in the different diets.

	Study 2				Study 3			
	Low carbohydrate diet		Fat reduced diet		Low carbohydrate diet		Fat reduced diet	
	planned	actual	planned	actual	planned	actual	planned	actual
Nutrient in Energy %								
Carbohydrate	30-35	37.2	55-60	45.2	≤20	29.0	≥50	41.7
Fat	35-40	36.8	<30	32.0	~55	38.2	≤30	30.1
Protein	25-30	21.2	~15	18.6	≥25	26.4	≤20	20.0
Total energy kcal/day	Deficit of 500	1949 ±555	Deficit of 500	1778 ±450	Deficit of 500	1470 ±500	Deficit of 500	1593 ±521

4.4 Statistics

Sample sizes were calculated according to study design. Descriptive statistics were used for mean (SD). Paired and independent samples *t*-tests were used to test for differences in and between groups when variables were normally distributed. Variables with a skewed distribution were log transformed or analyzed using non-parametric tests. Categorical variables were analyzed by Chi-square-tests. Correlations were calculated with Pearson's or Spearman's correlation coefficient. The percentage of change in variables was calculated for each subject and mean differences in percentage calculated from these. Statistical analyses were done with SPSS software. Specific statistical analyses used in the studies are described here.

Study 1. A mixed model analysis (analysis of variance [ANOVA] for crossover designs) was used to test for period, sequence or carryover effects. Differences between treatments were analyzed with a general repeated measures linear model. In paper 1 Bonferroni adjustment was used to control the overall α level, thus statistical significance was defined as a two-sided $P < 0.017$.

Study 2. In a generalized linear model two-way repeated measurement ANOVA, the interaction between time and diet or number of metabolic syndrome components using the Wilks lambda statistic was tested.

Study 3. In paper IV a repeated measures one-way ANOVA analysis was used. Intention to treat analysis for the primary endpoint (weight change) were calculated with baseline and last observation carried forward. These analyses were redone in a sensitivity analysis using multiple imputation analysis for missing body weights. In paper V a linear regression analysis was used to test for interaction in differences between subgroups.

5 Results

5.1 Study 1

Paper I

The polyunsaturated fatty acid rich control margarine reduced total cholesterol by 2.1% (95% CI −0.5, 4.7%) and LDL-cholesterol by 4.5% (95% CI 1.4, 7.6%) from screening values. Compared to the control margarine the tall sterol and rapeseed sterol margarines reduced total cholesterol by 6.8% (95% CI 4.0, 9.6%) and 6.5% (95% CI 4.2, 9.0%), LDL cholesterol by 9.0% (95% CI 5.5, 12.4%) and 8.2% (95% CI 5.2, 11.4%) and Apo B by 5.3% (95% CI 1.0, 9.6%) and 6.9% (95% CI 3.6, 10.2%), respectively. There were no significant changes in concentrations of HDL cholesterol, triglycerides, Apo A1 or lipoprotein (a). Apo B/A1 ratio was significantly reduced by the rapeseed sterol margarine.

Serum concentrations of α- and β-carotene and α-tocopherol were reduced by both sterol margarines. Concentrations of α-tocopherol were reduced more by the tall sterol versus the rapeseed sterol margarine. Concentrations of lycopene, β-tocopherol and phylloquinone were reduced only by the tall sterol margarine. After lipid-adjustment only concentrations of β-carotene were reduced by both sterol margarines. Adjusted α-tocopherol concentrations were reduced more by the tall sterol margarine than by the rapeseed sterol margarine.

Both sterol margarines increased concentrations of total, esterified, free and lipidadjusted sitosterol. The increase in free sitosterol was lower in the rapeseed sterol compared to the tall sterol period. Concentrations of total, esterified, free and lipidadjusted campesterol increased more by rapeseed sterol than by the tall sterol margarine. The change in total and free campesterol concentrations by tall sterol margarine was not significant. All the concentrations of brassicasterol were decreased by the tall sterol margarine but increased by the rapeseed sterol margarine.

Paper II

The rapeseed sterol margarine reduced E-selectin by 8.5% ($p = 0.012$) compared to the control margarine. Compared to the tall sterol margarine the rapeseed sterol margarine reduced E-selectin by 6.9% ($p = 0.037$) and tPAI-1 by 9.1% ($p = 0.008$). No significant differences were observed in TNFα and VCAM-1. There was no effect of the tall sterol margarine compared to the control margarine.

5.2 Study 2

Paper III

The calculated macronutrients and energy intake in the two dietary groups are shown in table 4. Weight loss after 3 months did not differ in the low-fat diet group (4.3 ± 4.0 kg; 4.4% ± 3.8%) compared to the low-glycemic-load diet group (4.9 ±3.4 kg (4.9% ± 3.2%). Serum concentrations of IL-6, TNF-α, leptin and PAI-1were reduced in both dietary groups, while concentrations of resistin were only reduced in the low-fat group. MCP-1 and adiponectin concentrations did not change. Changes in inflammatory biomarkers and adipokines did not differ between the dietary groups.

In the combined diet groups change in body weight was correlated with change in leptin concentration (Spearman $r = 0.358$; $P < 0.001$) but not with changes in other adipokines or inflammatory biomarkers. Baseline concentrations of IL-6 and resistin were higher and the adiponectin concentration was lower in subjects with metabolic syndrome compared to those with fewer than three components. Reductions in TNF-α and leptin concentrations were greater in subjects with full metabolic syndrome.

5.3 Study 3

Paper IV

The distribution of carbohydrates, fat and protein and total energy intake are shown in table 4. Intake of carbohydrates was ~13 percentage points lower in the low-carbohydrate compared to the fat-reduced group. Changes in total energy intakes did not differ between the two dietary groups; neither did energy expenditure due to physical activity.

Weight loss, changes in waist circumference, waist/hip ratio, and fat and muscle masses did not differ between dietary groups at the 4-week and 12-week post-TQD visits. Mean weight changes for the low-carbohydrate versus fat-reduced groups were -1.2 (SD 2.2) versus -0.5 (SD 2.0) kg, -0.2 (SD 3.3) versus 0.5 (SD 2.6) kg and 2.2 (SD 4.5) versus 2.1 (SD 3.9) kg at 4, 12 and 24 weeks post-TQD, respectively. Mean weight changes in continuous quitters were 0.0 (SD3.4) versus -0.5 (SD 3.3) kg for the low-carbohydrate versus fat-reduced diets, respectively, at the 12-week visit and 4.3 (SD 4.4) versus 3.0 (SD 4.0) kg at the 24-week visit.

Compared to participants in the fat-reduced group, participants in the low-carbohydrate group reported a higher total MNWS score during weeks 4 and 12 of treatment. The score for appetite increased more in the low-carbohydrate group, but the difference between diets did not reach significance (p=0.07).

Paper V

As changes in metabolic risk factors did not differ between dietary groups, we combined the groups to compare quitters to continuing smokers. Weight change was similar among 78 validated quitters as 30 continuing smokers (-0.1±3.0 kg vs 0.3±3.1 kg; p=0.7) as was change in waist circumference (-2.0±3.8 cm vs -0.9±3.9 cm; p=0.2).

Changes in triglyceride concentrations (-0.16±0.52 mmol/l vs 0.21±0.95 mmol/l; p=0.015) and diastolic blood pressure (-0.9±6 mmHg vs 1.9±8 mmHg; p=0.039) were more favorable in quitters. Changes in other cardiometabolic risk markers and HOMA-IR did not differ significantly between quitters and continuous smokers, nor did energy intake or RMR at week 4 post TQD. RMR was reduced by 3.9 ± 14.7% in quitters.

6 Discussion

The overall result in the present thesis is that several cardiometabolic risk factors may improve through dietary intervention in high risk individuals. The examined interventions were fat- or carbohydrate-restricted diets to reduce weight or plant sterol enriched margarines.

Weight gain after smoking cessation seemed to be counteracted by dietary follow-up and smokers following dietary advice when stopping smoking showed improvements in risk factors compared to non-quitters. The results were not different for a fat-reduced or a low-carbohydrate diet. The effects on inflammatory markers showed a similar pattern between a low-fat and a low-carbohydrate diet in a weight reduction study. Individual preferences may hence guide the choice of dietary strategy.

6.1 Methodological considerations and design issues

Study samples

All studies included adult men and women with slightly different age-limits. The upper limit of 65 years in the weight studies (study 2 and study 3) was chosen due to uncertainty about the appropriateness of weight loss interventions in older age [31]. The difference in BMI criteria for men and women in study 2 was set to have a larger proportion of men included as men are usually underrepresented in weight studies. This is reflected in the percentage of women in our studies which was 28% in study 1 (plant sterols), 58% in study 2 and 73% in study 3. Exclusion criteria were both for the safety of the participants and in order to exclude diseases, interventions or medication that could interfere with measurements and interpretation of results. In study 3 conditions in which the use of varenicline was not generally recommended when the study was planned, were excluded. The studies had very few participants not of European ethnicity (less than 2%) and the metabolic syndrome waist criterion for European populations was used for all.

Generalizability

The populations studied were clinically relevant, included free-living men and women at increased risk for CVD due to moderately elevated cholesterol levels or who were overweight or obese with an additional cardiometabolic risk factor as high blood pressure, high blood glucose, or dyslipidemia, or cigarette smoking. Volunteers tend to have better health than

comparable groups in the general population and the eligibility criteria excluded some disease categories. Furthermore, as most of the study participants were recruited by newspaper advertisement, they probably were more motivated for dietary changes and smoking cessation than people in the general population.

Study design

Strengths

The studies in this work were randomized controlled trials. The first study had a double blind design. The other studies compared different diets and could not be blinded. Study personnel in these studies were not blinded to treatment group due to lack of capacity, however most measurements were done with automatic devices or were laboratory analyses.

While dietary interventions for weight reduction can be challenging with high dropout rates, the studies generally had low loss to follow-up. Likewise, smoking cessation trials usually have low adherence. Smokers who are not able to quit have little motivation to continue participation to the end of the studies. In study 2 we had a complete dataset for inflammatory and adipokine markers at 3 months for 90% of participants and the 1-year completion rate was 81%. In study 3, 89% of the smokers completed 12 weeks and 78% attended the 24-week follow up (Table 3).

Limitations

The studies all had a short follow-up time. Adherence to dietary change is higher during short-term than longer studies [99]. However, short-term studies have the disadvantage of not being able to study clinical endpoints. Hard endpoint studies using dietary interventions need a great number of participants and many years of follow up requiring huge resources and costs and are not feasible in a small clinical research setting. The studies thus examine surrogate markers of disease. Not all surrogate markers are causal factors, as discussed above.

The plant sterol study (study 1) was designed principally to evaluate changes in lipid concentrations. A minimum of 2-3 weeks' intervention is sufficient to find changes in lipids [100]. Furthermore, plasma plant sterol concentrations are shown to be stabilized after 4 weeks [101], and the duration of the intervention has little influence on fat-soluble vitamin concentrations [102]. Follow-up in the dietary weight loss study (study 2) continued to 1 year and these results were published earlier [103]. As most weight loss usually appears during the first months of intervention, 3 months was considered an adequate time-point to examine the changes in adipokines and inflammatory markers. The same considerations may apply for

study 3, but naturally more long-time changes in weight and cardiometabolic parameters would have been of interest.

Another limitation is that of compliance in free-living subjects. In the plant sterol study compliance was evaluated by counting any leftover margarine in the returned boxes. Based on this measure compliance was good. The subjects were followed up by dieticians and instructed to make no dietary changes except changing the margarine. Body weight was stable as planned. In the weight studies the participants met with dietitians at regular visits to ensure compliance. In these studies a 7-day dietary record was filled out by the participants to estimate dietary intake and evaluate compliance. Underreporting is a well-known phenomenon in recall of food intake [104], but would be expected to be similar in randomized groups. The weight loss in study 2 and reduced weight gain in study 3 confirm that participants reduced caloric intake.

A further limitation was that differences between the reported diets were not as marked as planned. According to the food records the goals of macronutrients intake for the different diets were not completely reached. The low-carbohydrate groups did not restrict carbohydrate intake as much as planned. The gap between the planned and achieved carbohydrate intake in low-fat groups was more marked. The study participants might have been influenced by the popularity of the low-carbohydrate diets in recent years before the start of the studies. We noticed that some participants were disappointed to be randomized to the more traditional fat-reduced diets. Despite these weaknesses, the achieved differences in intake of macronutrients were clinically and statistically significant. The difference in fat intake between the diet groups was ~5 percentage points in study 2 and ~8 in study 3, the corresponding difference in carbohydrates was ~8 and ~13 percentage points (Table 4).

In regard to smoking, the validity of self-reported smoking depends on the social setting. We validated self-report with exhaled carbon monoxide (CO) concentrations. This measure reflects exposure to carbon monoxide in the previous day or two and does not exclude previous smoking. CO measurement is a simple and non-invasive test. The CO limit of 10 ppm has been commonly used, also in most studies of varenicline. An evaluation of different biochemical indicators concludes that CO $\geq$ 5 ppm optimally discriminate smokers from non-smokers in a city where smoking is banned in indoor public areas [105]. Cotinine, the main metabolite of nicotine has longer half-life and hence a longer window for detection. However, quantitative chromatography mass spectrometry analyses of cotinine are more expensive, have some false positives and the cut-off levels are not clear [105].

In study 2 and 3 a control group not given any dietary advice would have enabled us to evaluate the effect-size of interventions, not only comparing the two diets. However, a control group could have brought up some bias and ethical dilemmas. Control groups are often less motivated and less compliant. In study 3 this might have resulted in lower numbers successfully stopping smoking. We did not have statistical power to compare smoking cessation rates in the two dietary groups. To be able to detect a 10 % difference in quit rates between the groups, we would have needed almost 400 participants in each group to have 80% power at a 5% significance level.

6.2 Statistical considerations

The cross-over design of study 1 allowed for fewer participants. The effect of confounding covariates was reduced because each crossover subject acted as his or her own control. While the order in which the margarines were assigned could affect the outcome, this was unlikely in this study as adverse effects were not seen. The risk of carryover effect was reduced by the wash-out periods and order and carryover effects were tested for. The Bonferroni adjustment was applied in paper 1 because of the comparisons of three treatments.

In study 3 the sample size was calculated based on differences in body weight loss in earlier diet studies. No comparable estimates regarding dietary effects on weight gain after smoking cessation were available. Randomization was done in blocks to reduce the risk of possible imbalance due to different seasons, holiday periods and other factors. Additionally, balance was ensured in the case of not being able to include the planned number of smokers due to practical roadblocks or difficulties with recruitment. Intention-to-treat analyses were done not to break the randomization principle. Calculations for weight changes were done with baseline and last value carried forward and the effect of missing data was evaluated by a reanalysis with multiple imputing. We evaluated weight change according to various definitions of quitters, including point prevalence quitting with no cigarette during the last 7 days, continuous quitting with no cigarette at all since the quit date or Russell standard quitting that allows for 5 cigarettes since the target quit date. Which definition is best when it comes to evaluate change in weight after smoking cessation can be questioned, but not surprisingly continuous abstinence has been shown to lead to higher weight gain than point prevalence quitting [106].

6.3 Ethical considerations

All participants were well informed before study inclusion about the randomization procedures and about the importance of compliance to study program and visits. Informed consent emphasizes the participant's right to withdraw from the study without giving a reason. Study visits may be forgotten in a busy life and in studies involving lifestyle changes, visits may be missed due to lack of motivation especially if goals are not reached. Follow-up in such situations can be a balance between the principle of "nudging" and the needs of the study and on the other side the consent rights.

6.4 Discussion of each study

The study results are thoroughly discussed in each paper. In this paragraph the results are mostly reviewed in light of recent published research and the clinical implications are considered.

Study 1

The cholesterol-lowering effect of plant sterols was well known when the study was planned, while the effect of sterols derived solely from rapeseed oil had not been studied. One study compared sterols from tall oil to sterols from a 50/50 mix of tall oil and rapeseed oil and found equal LDL-cholesterol lowering effect [107]. A recent review showed that the carrier fat type in plant sterol products is of importance, rapeseed reduced total and LDL-cholesterol more than soybean/sunflower base [108]. However, our 3 test-margarines had the same base of 50/50 rapeseed and sunflower-oil, the control margarine reduced LDL cholesterol by 4-5% and the sterol margarines did not differ in lipid lowering effects.

The reduction in total and LDL-cholesterol of plant sterols and stanols are rather consistent. In contrast to the statins, plant stanols do not increase the Proprotein convertase subtilisin/kexin type 9 (PCSK9) concentration [77]. Some studies have reported a reduction in triglycerides that is more pronounced with higher baseline triglycerides and metabolic syndrome [77]. The participants in our study were not elected to have metabolic syndrome, mean baseline triglycerides was 1.26 mmol/L. Thus, we found no significant change in triglycerides. There are few data regarding plant sterols and lipoprotein (a). We found no change in line with earlier findings [77].

One of the concerns related to plant sterols/stanols is the negative effect on fat-soluble vitamins. A recent systematic review and meta-analysis of 41 RCTs (including study 1) concluded that consumption of plant sterols and plant stanols reduced lipid adjusted α- and β-carotene and lycopene. All values remained within normal range. The decrease was not considered to have deleterious health effects and could be counteracted by increasing intake of fruit and vegetables [109]. Our study showed significant reduction in lipid-adjusted β-carotene as well as some differences between the two sterol margarines. However, overall values remained within high normal limits. Few studies have evaluated the effect on vitamin K [109]. Our finding of decreased phylloquinone levels was not significant after lipid-adjustment.

A recent meta-analysis of studies regarding plant sterols/stanols and inflammation included our results, and concluded with no significant change in CRP or other inflammatory biomarkers [110].

Adding functional foods with plant sterols/stanols to usual diets may reduce the life-time cholesterol burden. Guidelines recommend plant sterols/stanols supplementation for individuals not qualifying for or not wanting pharmacotherapy, often younger people [21]. Another relevant group includes patients with side effects of statins. As plant sterols have additive lipid-lowering effect on top of statins and ezetimibe, they can be used together with pharmacotherapy to achieve desirable lipid levels [77]. Nutraceuticals are not reimbursed by the health care systems and hence must be paid for individually.

In our study only rapeseed sterol margarine reduced ApoB/A1 ratio significantly and had more favorable effects on fat-soluble vitamins and on markers of endothelial function and hemostasis compared to tall-sterol margarine. However, as far as we have been able to establish, a rapeseed derived plant sterol supplement has not yet been marketed. This is probably influenced by the EU limits for brassicasterol [111].

Study 2

More of the recent reviews and meta-analysis in the field of diet and inflammation have included the results of study 2 [112,113,114]. A meta-analysis of 10 studies comparing a variety of diets reported CRP lowering effect of low-fat diets in patients with metabolic syndrome, however this seemed to be dependent of weight reduction. No conclusion was drawn as to other inflammatory biomarkers [112]. Klemsdal et al found no difference in improvement of CRP between the low-fat and low-carbohydrate/low GI diet groups after 3 and 12 months [103]. Another review of GI/GL-diets concluded with benefits of low GI-diets

and suggested that conflicting results might be due to different intervention periods, the effects of low GI may be more pronounced in long-term studies [113]. Notably our study did compare low GL diet to a low-fat diet, not necessarily high in GI/GL. A meta-analysis of studies comparing high or low dietary fat found no association to adiponectin concentration [114].

The practical implication of our study is that even a modest weight reduction of less than 5% may decrease proinflammatory parameters in high risk groups of overweight and obese with metabolic syndrome criteria. The improvements did not differ between carbohydrate- and fat-restricted diets.

Study 3

Mechanisms behind the weight gain following smoking cessation are not fully understood. The degree of reduction in RMR is not consistent. We found a 3.9 % decrease in RMR 4 weeks after quitting, not significantly different from continuous smokers. This is in line with data from the large cross-sectional NEO study [115], but lower than earlier estimates and not enough to explain the lack of weight reduction according to reported dietary changes. A recent Australian cohort study concluded that the excess weight gain after 5 years in younger normal weight quitters was not explained by dietary changes or decrease in physical activity, in the general quitters tended to be healthier than continuing smokers [116]. Underreporting food and snack intake might be an explanation, as well as secondary changes to limit weight, but there still are some unanswered questions.

Some of the benefits of smoking cessation might be counteracted by weight gain and limiting this is of importance especially for overweight and obese smokers at risk for diabetes. A recent review [117] recommended further research: “combinations of weight- and tobacco-based treatments need to be tested with smokers who are at high risk for, or currently have, diabetes and those who have obesity.”

In our study insulin concentrations and HOMA-IR increased despite no weight gain in both quitters and continuous smokers after a quit attempt. The early changes in β-cell function and insulin resistance after smoking cessation are not fully elucidated. Other cardiometabolic factors improved in quitters and the sum of metabolic syndrome factors was not significantly changed.

According to our results, overweight and obese smokers trying to quit can follow a moderately carbohydrate- or fat-reduced diet and limit weight gain without impairing quit rates. However, a diet low in carbohydrates might be questioned in regard to smoking

cessation as this diet group reported higher appetite and total MNWS scores after quit date. This finding has been supported by other studies showing that quitters prefer more sweet tasting food [117].

6.5 Further research

Evidence-based and suitable nutraceuticals could be an important supplementation to other lifestyle changes in preventive medicine. The recommendations for use of plant sterol/stanol are weakened by lack of hard endpoint studies, such trials would of course demand multi-center engagement and huge resources. The proposed effects on dyslipidemia in metabolic syndrome are interesting. Our finding of the possible benefits of plant sterols from rapeseed oil should be investigated further.

Much research is done in the field of inflammation, however, today the inflammatory effects of diets and dietary patterns are still not consistent and further studies in different populations and patient groups are needed. As dietary changes may take time to be implemented, time to give stable biological effects, and also are difficult to sustain, long term studies may give results of more important clinical value.

Genetic knowledge has not yet shown to be useful in most clinical areas, but the research in this area is rapidly growing and may give a base for more individual dietary advices. Meanwhile personalized diets according to risk factors should be explored.

Though most weight gain is seen during the first months following smoking cessation, longer follow up is needed to ascertain if dietary intervention (and medical treatment) reduce or only delay the increase in weight. Our diet/smoking cessation study (study 3) indicated that restricting carbohydrates may increase withdrawal symptoms which could be an interesting question for further trials. There are some reports of changes in hormones, adipokines and inflammatory markers after quitting smoking, additional studies in this field may give more knowledge to the complexity of mechanisms behind postcessational weight gain and other metabolic changes.

7 Conclusions

7.1 Conclusions of each study

Study 1

Sterol margarines reduced atherogenic lipids and may have potential benefits on cardiovascular risk markers beyond LDL cholesterol reduction.

Margarines providing 2 g/day of sterols from rapeseed or tall oil resulted in similar reductions in LDL cholesterol and Apo B. Both sterol margarines reduced fat soluble vitamins, after lipid-adjustment only concentrations of β-carotene were reduced by both sterol margarines, adjusted α-tocopherol concentrations were reduced more by the tall-sterol margarine. Rapeseed-sterol margarine reduced E-selectin concentrations compared to the control margarine and tPAI-1 compared to the tall-sterol margarine.

Study 2

Hypocaloric diets leading to a mean weight loss of ~4%, improved inflammatory biomarkers and adipokines independently of dietary composition.

Energy restricted diets low in fat or low in glycemic load improved inflammatory biomarkers and adipokines similarly in overweight and obese individuals with components of metabolic syndrome. Serum concentrations of IL-6, TNF-a, PAI-1, and leptin were reduced in both dietary groups; while resistin concentrations were only reduced in the low-fat group. The improvements tended to be greater in subjects with full metabolic syndrome than in their counterparts with one or two components.

Study 3

A low carbohydrate and a fat-reduced diet showed similar effect on body weight in overweight and obese smokers trying to quit and some metabolic risk factors were improved in quitters.

Energy restricted diets low in carbohydrate or fat did not differ in effects on body weight, fat mass or body circumferences in overweight and obese smokers trying to quit, but the low carbohydrate diet group reported higher withdrawal symptoms. The diets seemed to ameliorate early weight gain without decreasing quit rates (in comparison to results in previous studies). Quitters showed no increase in weight and improvement in triglycerides

and diastolic blood pressure after 12 weeks compared to continuing smokers. Changes in other cardiometabolic risk factors and homeostasis assessment model insulin resistance (HOMA-IR) did not differ between quitters and continuous smokers. We found a 3.9 % decrease in RMR 4 weeks after quitting,

7.2 Concluding summary

Nutraceuticals may be an important supplementation to dietary intervention, we report improved lipids and inflammatory parameters in hypercholesterolemia adults after intake of plant sterol enriched margarines.

In overweight and obese men and women energy restricted diets low in carbohydrate or low in fat similarly improved inflammatory biomarkers and adipokines and did not differ in the effect on weight-gain and body composition in smokers trying to quit. Quitters showed improvement in some metabolic risk factors.

In summary this work together indicates that short time dietary interventions could improve traditional risk factors as well as markers of inflammation in individuals at increased risk for cardiovascular disease. Effects were on the whole not dependent on whether the interventions were low-fat or low-carbohydrate diets.

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9 Appendix

KOST OG RØYKESLUTT

Minnesota Nicotine Withdrawal Scale

0 = slett ikke 1 = litt 2 = moderat 3 = veldig mye 4 = ekstremt mye

1. Trang til å røyke ☐
2. Nedtrykt humør ☐
3. Irritasjon, frustrasjon eller sinne ☐
4. Angst og uro ☐
5. Konsentrasjonsvansker ☐
6. Rastløshet ☐
7. Økt matlyst ☐
8. Vansker med å falle i søvn ☐
9. Vansker med å sove hele natten ☐

Poengsum: _____