Effects of targeted substitution dietary guidelines on dietary intake and ischaemic heart disease risk factors in an adult Danish population: The DIPI randomised controlled trial

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Effects of targeted substitution dietary guidelines on dietary intake and ischaemic heart disease risk factors in an adult Danish population:

The DIPI randomised controlled trial

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Preface

This PhD thesis is based on a randomised controlled trial conducted during my employment at the Technical University of Denmark, National Food Institute (DTU Food), from November 2012 to March 2018, including two maternity leaves. I was responsible for the design and planning of the trial, including applying to the relevant ethical committee. Furthermore, I planned and took part in the data collection and contributed to the data processing. Finally, I did the data analysis and the writing of the included paper and manuscripts.

The trial is a part of the project "Diet and prevention of ischemic heart disease: a translational approach" (DIPI, www.dipi.dk), which is supported by the Danish Council for Strategic Research (Today Innovation Found Denmark) (Contract 0603-00488B). The PhD scholarship was further co-funded by the Technical University of Denmark, National Food Institute.

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A very special thanks to my co-workers at the PhD office – Lea, Sofie, Ida, Maria, Nanna and Ellen and to Mette and Nina who was there when I started my PhD – I appreciate all of our conversations, both the professional ones and the personal ones. To my co-workers in the research group for risk benefit for introducing me to the research field of risk-benefit assessment.

I am very grateful to my family and friends for your endless support and cheering, and special thanks goes out to my mom and Michael for helping out in so many ways and for generally making life much easier for a busy family with small children. Also thanks to my parents in law, Jan and Bente, for almost "moving in" and for taking extra good care of the kids when Jesper and I needed to pitch into work.

Thanks to my dear boyfriend Jesper for your love and rock steady support during this process, and last but certainly not least – thank you Otto and Ellen for always reminding me of what really matters in life!

List of papers

This thesis is based on the following three papers, hereafter referred to as paper I, paper II, and paper III:

I) Johanne L. Arentoft, Camilla Hoppe, Elisabeth W. Andersen, Kim Overvad, Inge Tetens. Associations between adherence to the Danish Food-Based Dietary Guidelines and cardiometabolic risk factors in a Danish adult population: the DIPI study [Br J Nutr]

II) Johanne L. Arentoft, Camilla Hoppe, Rikke Andersen, Elisabeth W. Andersen, Rikard Landberg, Kim Overvad, Inge Tetens. Short and long term dietary effects of applying Substitution dietary guidelines and Official dietary guidelines in a Danish adult population: The DIPI randomised controlled trial. [Submitted]

III) Johanne L. Arentoft, Rikke Andersen, Elisabeth W. Andersen, Kim Overvad, Inge Tetens, Camilla Hoppe. Effects of targeted substitution dietary guidelines on ischemic heart disease risk factors in an adult Danish population: The DIPI randomised controlled trial [Submitted]

A printed version of paper I and the manuscripts of papers II and III are included in appendices.

Summary

Background and aim: Ischaemic heart disease (IHD) is the leading cause of morbidity and mortality worldwide with an estimated 7.4 million deaths due to IHD in 2015. Addressing modifiable risk-factors such as diet can help prevent IHD.

The overall aim of this thesis was to study the effects of targeted substitution dietary guidelines on dietary intake and IHD risk factors in an adult Danish population. The specific research objectives were: i) to investigate the associations between adherence to the current Danish official dietary guidelines assessed by a diet quality index (DQI) and selected cardio-metabolic risk factors (paper I); and ii) to examine the short- and long term effects of applying targeted substitution dietary guidelines on dietary intake (paper II) and IHD risk factors (Paper III).

Methods: A 6-month single-blinded parallel randomised controlled trial (RCT) with a 6-month follow-up was conducted in a real-life setting including an adult Danish population with a minimum of one self-reported risk factor of IHD. At baseline participants were assigned to either a control group advised to follow their habitual diet or one of two intervention groups receiving either targeted substitution dietary guidelines or the Danish official dietary guidelines.

At baseline and after 6 and 12 months, information on dietary intake of the participants was obtained by a 7-day web-based dietary record, and a DQI score was calculated as a marker for adherence to the two sets of dietary guidelines. Fasting blood samples were analysed for lipid- and glyceamic biomarkers and alkylresorcinols, and blood pressure, heart rate, anthropometric measurements, and background questionnaires were collected. Linear regression analyses were applied.

Results: A total of 222 participants were enrolled with a median age of 51 years, 59% were women, and 73 % were overweight or obese.

In the cross-sectional study (paper I) based on the baseline data of the RCT, the DQI score was significantly inversely associated with low-density lipoprotein cholesterol/high-density lipoprotein cholestreol (HDL-c) -ratio and triglycerides and positively associated with HDL-c. For men, DQI was inversely associated with body mass index, trunk fat, high-sensitivity C-reactive protein, haemoglobin A_{1c}, insulin, and insulin resistance. In women, DQI was positively associated with systolic blood pressure.

The overall results of the RCT (paper II and paper III) showed that compared with the habitual diet group, the group following the targeted substitution dietary guidelines statistically significantly increased their intake of whole grains, dietary fibre, and fine vegetables, and statistically significantly decreased their percentage of energy (E%) intake from saturated fat (SFA) from baseline to 6 months. In the same period the group following the Danish official dietary guidelines statistically significantly decreased their E% intake from SFA compared with the habitual diet group. From baseline to 12 months, both intervention groups statistically significantly increased their intake of whole grains and fish, and the group receiving the Danish official dietary guidelines still showed a statistically significantly decrease in E% intake from SFA compared with the habitual diet group. Additional analysis showed that from baseline to 6 and 12 months the DQI score statistically significantly increased in both intervention groups compared to the habitual diet group. No overall statistically significantly differences in change in cardio-metabolic risk factors were found in either of the two intervention groups compared to the habitual diet group. In addition, self-reported whole grain intake was associated with plasma alkylresorcinol concentrations at baseline.

Conclusion: Closer adherence to the current Danish official dietary guidelines, assessed by a DQI, was associated with a more beneficial cardio-metabolic risk profile in a Danish adult population with at least one self-reported risk factor for IHD (paper I).

In the short-term the targeted substitution dietary guidelines were more effective than the Danish official dietary guidelines in changing the diet, resulting in a dietary composition of the overall diet being more cardio-protective compared with the habitual diet. The long-term effectiveness of the two sets of dietary guidelines was similar (paper II). This was supported by the observed short and long term increase in DQI score, indicating increased adherence to both sets of dietary guidelines.

However, neither the targeted substitution dietary guidelines nor the Danish official dietary guidelines showed any overall beneficial effects on IHD risk factors compared with the habitual diet (paper III).

The findings of the three papers included in this thesis will add to the understanding of the impact of applying dietary guidelines on dietary intake and IHD risk factors and will be of importance in future revisions of dietary guidelines.

Sammendrag

Baggrund og formål: Iskæmisk hjertesygdom (IHS) er den førende årsag til morbiditet og dødelighed i verden med omkring 7,4 millioner dødsfald som følge af IHS i 2015. Fokus på modificerbare risikofaktorer så som kost kan hjælpe med at forebygge IHS.

Det overordnede formål med denne afhandling var at undersøge effekterne af målrettede substitutionskostråd på kostindtag og risikofaktorer for IHS i en voksen dansk befolkning. De specifikke forskningsmål var: i) at undersøge sammenhængen mellem efterlevelse af de nuværende officielle danske kostråd, vurderet ved hjælp af et kostindex (DQI), og udvalgte kardiometaboliske risikofaktorer (artikel I), og ii) at undersøge de kort- og langsigtede effekter af målrettede substitutionskostråd på kostindtag (artikel II) og risikofaktorer for IHS (artikel III).

Metoder: En 6-måneders enkeltblindet, parallelt randomiseret kontrolleret undersøgelse (RCT) med en 6-måneders opfølgning blev gennemført i en "real-life setting" i en voksen dansk befolkning med mindst én selvrapporteret risikomarkør for IHS. Ved baseline blev deltagerne randomiseret i enten en kontrolgruppe, der blev henvist til at følge deres sædvanlige kost, eller til en af to interventionsgrupper, der enten modtog målrettede kostråd eller de officielle danske kostråd.

Ved baseline og efter 6 og 12 måneder blev information om kostindtaget blandt deltagerne indsamlet ved hjælp af en 7-dages, web-baseret kostdagbog, og en DQI-score blev beregnet som en markør for efterlevelse af kostrådene i hver interventionsgruppe. Fastende blodprøver blev taget og analyseret for lipid- og glykæmiske biomarkører samt alkylresorcinoler. Blodtryk, puls, antropometriske målinger og baggrundsspørgsmål blev indsamlet, og lineære regressionsanalyser anvendt.

Resultater: I alt blev 222 deltagere inkluderet med en median alder på 51 år, hvoraf 59% var kvinder, og 73% var overvægtige eller fede.

I tværsnitsstudiet (artikel I) baseret på baseline data fra RCT'en var DQI-scoren signifikant invers associeret med lavdensititets lipoprotein kolesterol/højdensititets lipoprotein kolesterol (HDL-c)-ratio og triglycerid og positivt associeret med HDL-c. For mænd var DQI-scoren omvendt associeret med body mass index, kropsfedt, high sensitivity C-reaktivt protein, haemoglobin A_{1c}, insulin og insulin resistens. Hos kvinder var DQI positivt associeret med systolisk blodtryk.

De samlede resultater af RCT'en (artikel II og artikel III) viste, at gruppen, der modtog målrettede substitutionskostråd, statistisk set signifikant øgede deres indtag af fuldkorn, kostfibre og fine grøntsager og statistisk set signifikant sænkede deres procentdel af energi (E%) indtaget fra mættet fedt statistisk set signifikant sammenlignet med den habituelle kostgruppe, fra baseline til 6 måneder. I samme periode sænkede gruppen, der modtog de officielle danske kostråd, E% -indtaget fra mættet fedt statistisk set signifikant sammenlignet med den habituelle kostgruppe. Fra baseline til 12 måneder forøgede begge interventionsgrupper deres indtag af fuldkorn og fisk statistisk set signifikant, og gruppen, der modtog de officielle danske kostråd, sænkede deres E% indtag fra mættet fedt sammenlignet med den habituelle kostgruppe. Yderligere analyser viste, at fra baseline til både 6 og 12 måneder steg DQI-scoren statistisk set signifikant i begge interventionsgrupper sammenlignet med den habituelle kostgruppe.

Der blev ikke fundet nogen overordnede statistisk set signifikante forskelle i ændringer i kardiometabolske risikofaktorer i nogen af interventionsgrupperne sammenlignet den habituelle kostgruppe.

Hertil kommer, at selvrapporteret fuldkornsindtag var associeret med plasma alkylresorcinoler ved baseline.

Konklusion: Bedre efterlevelse af de nuværende danske officielle kostråd, vurderet ved et DQI, var forbundet med en mere fordelagtig kardiometabolisk risikoprofil i en voksen dansk befolkning med mindst en selvvurderet risikofaktor for IHS (artikel I).

På kort sigt var de målrettede substitutionskostråd mere effektive til at ændre kosten end de officielle danske kostråd, hvilket resulterede i, at kostsammensætningen af den samlede kost var mere hjertebeskyttende end kosten i den habituelle kostgruppe. De langsigtede effekter af de to sæt kostråd var sammenlignelige (artikel II). Dette blev understøttet af den observerede kort- og langsigtede forøgelse i DQI-score, hvilket indikerer en øget efterlevelse af begge sæt kostråd.

Dog viste hverken de målrettede substitutionskostråd eller de officielle danske kostråd generelle gavnlige effekter på risikofaktorer for IHS sammenlignet med en habituel kost (artikel III).

Resultaterne af de tre artikler, der indgår i denne afhandling bidrager til forståelsen af effekten kostråd på kostindtaget og IHS-risikofaktorer, og bør have betydning for eventuelle revideringer af kostrådene.

Abbreviations

HEI:	American Healthy Eating Index
BMI:	Body mass index
CVD:	Cardiovascular disease
CV:	Coefficient of variation
CI:	Confidence interval
CHD:	Coronary heart disease
CRP:	C-reactive protein
DBP:	Diastolic blood pressure
DASH:	Dietary Approaches to Stop Hypertension
DQS:	Dietary Quality Score
DIPI:	Diet and prevention of ischemic heart disease: a translational approach
DQI:	Diet Quality Index
E%:	Energy percentage
FFQ:	Food Frequency Questionnaire
HbA_{1c}:	Haemoglobin Ab_{1c}
HR:	Hazard ratio
HDL:	High-density lipoprotein
hsCRP:	High-sensitivity C-reactive protein
hsCRP:	High-sensitivity CRP
HOMA:	Homeostatic model assessment
HOMA-IR:	Homeostatic model assessment-insulin resistance
IHD:	Ischaemic heart disease
LDL:	Low-density lipoprotein
MUFA:	Monounsaturated fatty acids
MI:	Myocardial infarction
PUFA:	Polyunsaturated fatty acids
RCT:	Randomised controlled trial
SFA:	Saturated fatty acids
SBP:	Systolic blood pressure
T:	Tertile
TAG:	Triglycerides
UK:	United Kingdom
VLDL:	Very low-density lipoprotein

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1 Introduction

1.1 Rationale for this thesis

The research described in this PhD thesis concerns the impact of targeted substitution dietary guidelines for the prevention of the most common cardiovascular disease (CVD), namely ischaemic heart disease (IHD).

IHD arises from a combination of genetic and behavioural risk factors. Addressing behavioural risk factors, such as an unhealthy diet, may help improve some of the clinical conditions contributing to the development of IHD, including hypertension, diabetes, hyperlipidaemia, overweight and obesity [1–4]. Therefore, identifying the optimal diet for prevention of IHD in the general population is crucial.

Dietary guidelines worldwide, including the Danish official dietary guidelines, are typically based on systematic literature reviews of the association between food intake and different relevant diet-related diseases in the target group of the recommendations [5–7]. However, targeted dietary guidelines addressing one specific nutrition-related lifestyle disease, such as IHD, might be even more effective in preventing IHD than dietary guidelines targeting all relevant nutrition-related diseases, as in the case of the Danish official dietary guidelines.

Previously, dietary research has focused mainly on single foods or nutrients to evaluate the association between diet and disease outcomes. Yet this does not resemble real-life situations as people consume foods and nutrients in combination. During recent decades, therefore, research on diet–disease associations have focused on the overall quality of dietary patterns and national dietary guidelines [8–11]. However, the optimal intake of foods and nutrients in combination to prevent IHD is still unclear.

When individuals change their intake of specific nutrients or foods, they primarily change their dietary composition rather than their total energy intake [12]. Therefore, the changes that individuals must make toward achieving a healthy dietary pattern are important, and the foods or nutrients used by individuals as substitutes, to achieve a healthy dietary pattern, are not insignificant; indeed, this substitution aspect is important.

1.2 Aim and objectives

The overall aim of this thesis research was to study the effects of targeted substitution dietary guidelines on dietary intake and IHD risk factors in an adult Danish population.

The specific research objectives were:

- To investigate the associations between adherence to the current Danish official dietary guidelines, assessed by a diet quality index (DQI), and selected cardiometabolic risk factors (paper I).
- To examine the short- and long-term effects of applying targeted substitution dietary guidelines on dietary intake (paper II) and IHD risk factors (paper III).

The three manuscripts (paper I, paper II, and paper III) are included in this thesis as **Appendix A**.

This PhD thesis is an integrated part of the large-scale research project entitled ´Diet and prevention of ischemic heart disease: a translational approach´ (DIPI; www.dipi.dk).

The aim of DIPI was to study dietary patterns and optimal substitutions of energy-providing foods and macronutrients in relation to IHD development through observational studies, and to further translate this knowledge into food-based dietary guidelines targeting prevention of IHD, to be tested in a randomised controlled trial (RCT).

Therefore, the focus of the research discussed in this thesis is the effects of food-based dietary guidelines on dietary intake and intermediate risk factors for IHD in a dietary RCT.

2 Background

2.1 Ischaemic heart disease

2.1.1 Definition

CVDs are the general term used for a group of disorders of the heart and blood vessels, including IHD [13]. IHD is caused by inadequate oxygen as a result of reduced blood supply to the heart, often caused by atherosclerosis which causes narrowing or blocking of the coronary arteries [13, 14]. The main clinical manifestations of IHD are angina pectoris (chest pain or discomfort) and myocardial infarction (MI).

IHD is sometimes referred to using other terms, such as coronary heart disease (CHD), coronary artery disease, and atherosclerotic heart disease. In this thesis, the term IHD is used to refer to IHD in general; when referring to previous studies on IHD and CVD risk factors or other subcategories of CVDs, the term used by the authors will be reported.

2.1.2 Risk factors

IHD arises from a combination of unmodifiable and modifiable behavioural risk factors (**Table 1).** Modifiable behavioural risk factors include an unhealthy diet, smoking, high alcohol intake, and sedentary lifestyle [13, 15]. Addressing these behavioural risk factors can help improve various clinical conditions including hyperlipidaemia, hypertension, overweight/obesity, and diabetes, which all contribute to the progression of atherosclerosis and therefore are major risk factors for the development of IHD [1–4].

As serum lipids play an important role in the pathogenesis of atherosclerosis (see section 2.1.3), hyperlipidaemia is one of the most important risk factors for development of IHD. Hyperlipidaemia is defined as an abnormal lipid profile with elevated concentrations of total cholesterol, low-density lipoprotein (LDL), cholesterol or triglycerides (TAG), or low levels of high-density lipoprotein (HDL) cholesterol [4]**.** In addition, C-reactive protein (CRP) serves as a marker for inflammation, which is present in all stages of atherosclerosis. Thus, managing CRP levels is a supportive measure for addressing IHD risk in clinical practise [16, 17].

Hypertension or high blood pressure includes both measures of systolic blood pressure and diastolic blood pressure. Overweight/obesity is defined according to body mass index (BMI) and abdominal obesity, which is defined by measuring the waist circumference [4, 18].

Diabetes or prediabetes are terms used to describe a metabolic disorder characterized by chronic hyperglycaemia and impaired insulin secretion and or insulin action [19, 20]. Haemoglobin A_{1c} (HbA_{1c}) levels serve as a measure of an individual's average glucose level over the previous 3 months [21]. In addition, the homeostatic model assessment (HOMA) is widely used as a measure of changes in insulin resistance (HOMA-IR) that may increase the risk of development of diabetes and prediabetes [22, 23].

In **Table 1,** unmodifiable and modifiable behavioural risk factors are collected and presented together with the recommended cut-off levels for defining the above-mentioned risk factors of IHD in clinical practise.

Table 1 Unmodifiable and modifiable behavioural risk factors and recommended cut-off levels for the definition of risk factors for IHD in clinical practice.

Unmodifiable risk factors	Modifiable risk factors	Clinical conditions accelerating atherosclerosis and definitions in clinical practise †	
Genetics Age Sex	Unhealthy diet Smoking Excess alcohol intake Sedentary lifestyle	Hyperlipidaemia	Total cholesterol > 5.0 mmol/L LDL-c > 3.0 mmol/L HDL-c: men < 1.0 mmol/L women < 1.2 mmol/L TAG level > 1.7 mmol/L
		Hypertension	SBP > 140 mmHg DBP > 90 mmHg
		Overweight and obesity	BMI ≥ 25 kg/m^2 and BMI > 30 kg/m^2
		Abdominal obesity	WC: men > 94 cm women > 88 cm
		Prediabetes and diabetes	Glucose > 7.0 mmol/L HbA_{1c} > 6.5% HOMA-IR > 2.29 §

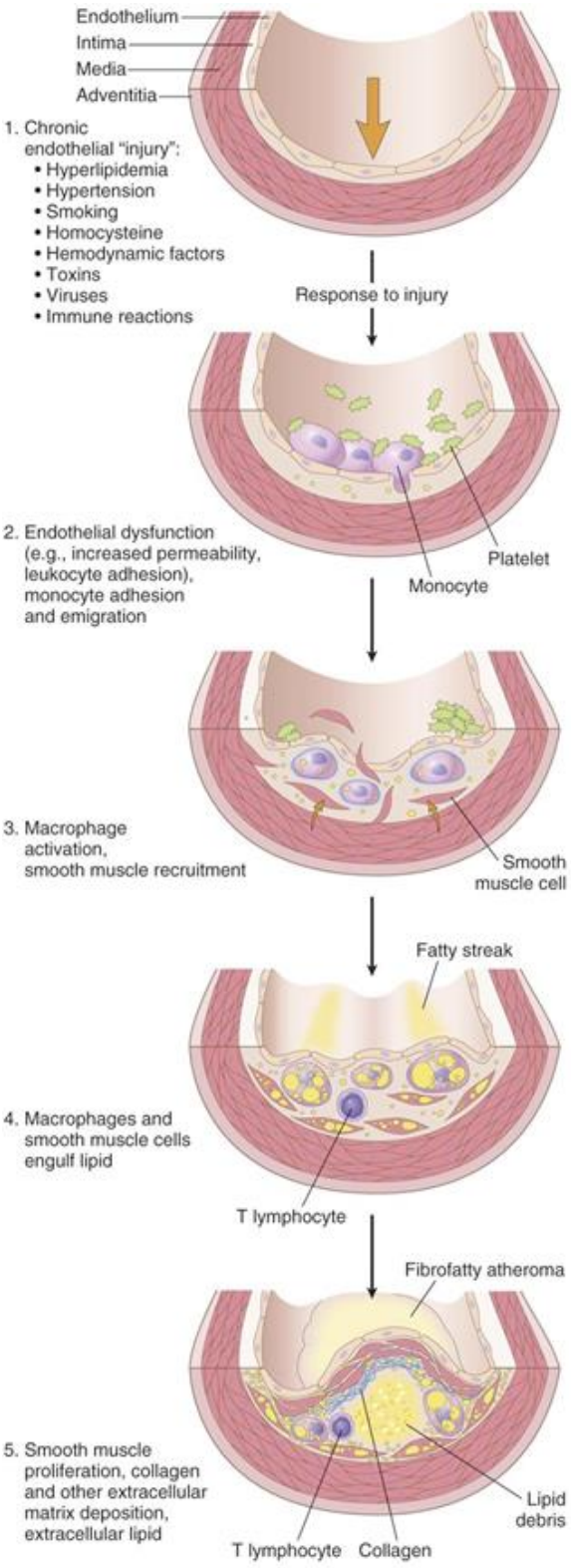
Abbreviations: IHD, ischaemic heart disease; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TAG, triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; HbA_{1c}, haemoglobin A_{1c}; HOMA-IR, homeostatic model assessment insulin resistance.

† According to European guidelines on cardiovascular disease prevention in clinical practice and the World Health Organization [4, 19].

‡ A cut-off point for insulin resistance measured by HOMA-IR does not exist; however, a definition for low insulin resistance has been suggested as HOMA-IR > 2.29 [24].

2.1.3 Pathogenesis

The primary cause of IHD is atherosclerosis, which is a slowly progressing arterial disease (**Figure 1**) [25].



The smooth layer of the artery wall that faces the arterial lumen is mainly made up of endothelial and smooth muscle cells, which serve as a semi-permeable barrier where fluid, nutrients, and so on can be transported from the blood to the tissues [14].

Briefly, the evolution of atherosclerosis is based on the 'The Response to Injury' theory, and begins with endothelial injury [14]. Endothelial injury results from different internal or external insults, including haemodynamic disturbances, e.g. where arteries branch and development of vascular disorders such as hypertension, hyperlipidaemia, or chronically elevated blood glucose levels [14].

Endothelial injury results in secretion of cytokines, causing a chronic inflammatory response. Furthermore, endothelial injury causes endothelial dysfunction with increased permeability where adhesion and migration of monocytes, smooth muscle cells, and lipoproteins (LDL and very low-density lipoprotein; VLDL) takes place [14, 25]. Inside the intima, monocytes differentiate into macrophages and take up the LDL, which has undergone oxidation. The macrophages continue to take up oxidized LDL and eventually turn into ´foam cells´, until apoptosis occurs and the foam cells die. After

Figure 1 Progression of changes in the arterial wall in response to injury. 1: Normal arteries, 2: Endothelial injury with adhesion of monocytes, 3: Migration of monocytes and smooth muscle cells (SMCs) into the intima, 4: Macrophage and SMC uptake of lipids, 5: Well-developed plaque. Illustration from Kumar et al. [14].

apoptosis, the lipids will accumulate, and thickening of the intima eventually creates fatty streaks, which can evolve into atherosclerotic plaques and cause reduced blood flow, resulting in oxygen inadequacy [14].

Serum lipids play an especially important role in the pathogenesis of atherosclerosis because of the accumulated uptake by macrophages in the intima [25, 26]. In addition, the increased oxidization of LDL inside the intima further stimulates release of cytokines, chemokines, and growth factors, resulting in increased recruitment of monocytes to the endothelial injury, leading to accelerated atherosclerosis plaque development [14, 26].

2.1.4 IHD from a public health perspective

As in most other Western countries, IHD is the leading cause of morbidity and mortality in Denmark. There were an estimated 7.4 million deaths worldwide owing to IHD in 2015 [13]. In Denmark, the total incidence of IHD in 2015 was about 18,500, with a total prevalence of approximately 100,000 men and around 61,000 women [27]. In total, IHD accounts for over 7% of all deaths in Denmark, making IHD one of the deadliest diseases [28].

Despite this, the number of deaths due to IHD in Denmark and most other European countries has decreased during the period from 1995 to 2015 [27, 28]. The decreased incidence of IHD, together with increasing survival of individuals with the disease, have contributed to the decrease in mortality [29]. However, increased survival of IHD also increases the need for health care services for people living with the disease, resulting in a total annual cost for treatment and care of 1.760 billion DKK [28].

2.2 Evidence-based dietary guidelines

To promote health and reduce the risk of diet-related diseases, including IHD, in the general population through improved nutrition and physical activity, health authorities in Denmark and most other Western countries have established dietary guidelines [6, 7, 30–32]. These guidelines are based on the available body of scientific evidence on the association between food intake and physical activity and different diet-related diseases [5–7, 33, 34].

In Denmark, the first set of dietary guidelines was launched in 1970, and the guidelines have been continuously revised since. The latest revision of the Danish official dietary guidelines was published in 2013, based on a systematic literature update of the scientific literature on the

associations between food intake and physical activity and different diet-related diseases and the Nordic Nutrition Recommendations 2012 [5, 35]. The official dietary guidelines contain 10 recommendations for foods and physical activity (**Table 2**) [36]. The purpose of the Danish official dietary guidelines is to translate the nutrient requirements of the population into dietary recommendations, which requires consideration of the usual Danish dietary intake and physical activity level as well as Danish food culture and availability [5]. However, adherence to the Danish official dietary guidelines remains low, with around 90% of Danish adults who do not eat the recommended daily amount of fruit and vegetables and 82% who do not eat the recommended daily amount of fish [37, 38].

Table 2 The ten Danish official dietary guidelines [36].

Danish official dietary guidelines
Eat a variety of foods, but not too much, and be physically active
Eat fruits and many vegetables (600 g/10MJ/day) †
Eat more fish (350 g/week)
Choose whole grains (min 75 g/10MJ/day)
Choose lean meats and cold meats (max 500 g/week)
Choose low-fat dairy products
Eat less saturated fat
Eat foods with less salt
Eat less sugar
Drink water

† At least half should be vegetables [5].

2.2.1 Promoting dietary guidelines

Many initiatives have been implemented in the past decade to promote the Danish official dietary guidelines among the general population, to facilitate making healthier choices. These initiatives include partnerships, such as the Keyhole and the Whole Grain partnerships [39, 40]. Both aim to make it easier for consumers to find and choose healthier foods and to follow the national dietary guidelines. Among other approaches, this is done through food labelling, ensuring that foods labelled with the Keyhole or Whole Grain logo meet specific requirements for whole grain, fibre, fat, sugar, and salt content.

2.2.2 Targeted dietary guidelines and rationale of including specific substitutions

The evidence base for the Danish official dietary guidelines presents a convincing or probable causal relationship between a lower risk of IHD and CVD and the intake of fish, fruit and vegetables, dietary fibre, whole grains, and the substitution of saturated fat with polyunsaturated fat [5]. These foods and nutrients were the main focus when developing the five targeted dietary guidelines investigated in this thesis research.

These targeted substitution dietary guidelines were further developed with recommendations for specific substitutions, as it is well established that when individuals change their intake of specific nutrients or foods, they primarily change their dietary composition rather than their total energy intake [12]. Therefore, the shifts that individuals need to make toward achieving a healthy dietary eating pattern are important, and the specific foods or nutrients used as substitutes by individuals to achieve a healthy dietary pattern are crucial. Therefore, the rationale in this study for establishing specific substitutions was to guide and motivate participants' changes in foods or nutrients toward those that are the most cardioprotective.

2.3 Foods and nutrients relevant to IHD prevention

The current Danish official dietary guidelines are based on a systematic literature update of the scientific literature up to 15 October 2012, which, as mentioned, showed a convincing or probable causal relationship between the intake of fish, fruit and vegetables, dietary fibre, whole grains, substitution of saturated fat with polyunsaturated fat, and lower risk of IHD and CVD [5].

In addition, the author of this thesis conducted literature searches in PubMed on the selected food groups or nutrients, including substitutions, and IHD, from October 2012 to January 2018. Reference lists of the papers identified in these searches were read through, to search for additional relevant articles.

The focus of the literature search was primarily meta-analyses. Thus, in the following paragraphs recent relevant meta-analyses, including RCTs and observational studies as well as selected supplementary single cohort studies, all aiming to investigate the above-mentioned foods and nutrients, including red meat (owing to its high-saturated fat content) in relation to IHD will be presented.

2.3.1 Fish

In this thesis and the included papers, fish refers to fresh, frozen, smoked, or canned fish and shellfish. Fish contains n-3 polyunsaturated fatty acids (PUFAs) and is an important source of vitamin D and selenium. The cardioprotective effect of fish consumption has been suggested to be mainly ascribable to the content of n-3 fatty acids [5].

The suggested cardioprotective effects of n-3 PUFAs include modulation of serum lipids, regulation of blood pressure, reducing arrhythmia (irregular heartbeat), endothelial function

improvement, and inhibiting inflammation, thereby producing a protective effect against diseases characterised by low-level chronic inflammation, such as diabetes and obesity [41–43].

In two recent meta-analyses of prospective cohort studies and case-control studies, an association between fish intake and lower risk of IHD and heart failure were observed [44, 45]. Furthermore, a recent prospective cohort study suggested that replacing red meat, poultry, or lean fish with fatty fish was associated with a lower risk of MI [46]. This is supported by results based on the same cohort, where a 12% lower relative risk of MI in men was found when the highest and lowest quintiles of fatty fish intake were compared; in addition, a 22% lower risk was found in women [47]. In a recent review of clinical trials, fish consumption had a positive effect on relevant risk factors, such as TAG, HDL, and platelet aggregation [48].

Nevertheless, some inconsistencies exist in the findings regarding fish consumption and risk of IHD. In two studies, including three cohort studies, no association was found between fish intake and IHD risk [49, 50]; these results are supported by those of another study in which fish, instead of red meat, was not associated with a lower risk of CHD [51].

2.3.2 Red meat

In this thesis and the included papers, red meat refers to beef, veal, pork, lamb, and offal. Red meat also includes both raw and processed meat, the latter often preserved by salting, smoking, or by the addition of other preservatives such as nitrates [5, 37].

Red meat consumption comprises intake of important nutrients like protein, B-vitamins (B_{12} and B_6), iron, zinc, and selenium. However, the potential adverse effects of consuming red meat and processed meats involve large intakes of saturated fat (SFA), cholesterol, heme iron, and sodium, all of which have been suggested to produce harmful effects on serum lipids, oxidative stress, inflammation, and blood pressure [43, 52, 53].

There are inconsistencies in the recent literature regarding the association between red meat consumption and IHD. However, processed meats have been constantly associated with IHD. In a meta-analysis of prospective cohorts, processed meat consumption was associated with a higher risk of CHD, and unprocessed meat consumption was associated with a higher increase or no risk [52]. This was supported by another meta-analysis of prospective cohort studies suggesting that a high consumption of red meat, especially processed red meat, was associated with the risk of stroke [54]. However, no association between consumption of red or processed

meat and IHD mortality was found in a meta-analysis of prospective cohort studies, although consumption of both red and processed meat was associated with CVD [55].

2.3.3 Dietary fat

Dietary fat includes different fatty acids such as SFA and *trans* fatty acids that, as previously described, are primarily found in animal products, such as meat, monounsaturated fatty acids (MUFAs), and PUFAs, and that (in addition to the aforementioned n-3 fatty acids found primarily in fish) also include n-6 PUFAs, found primarily in vegetable oils and nuts [56].

Recent results from meta-analyses including RCTs and cohort studies provide strong evidence that replacing SFA with PUFAs is beneficial for cardiovascular health [43, 57–59]. A systematic review including regression analysis of 84 RCTs found that when 1 E% (energy percentage) from SFA was replaced with an equal amount of PUFAs, reductions were seen in total cholesterol, LDL cholesterol, HDL cholesterol, TAG, the LDL cholesterol to HDL cholesterol ratio, and TAG to HDL cholesterol ratio [57]. In addition, a meta-analysis including 13 prospective cohort studies concluded that SFA should be replaced with n-6 PUFAs in the recommendations for primary prevention of CHD [58]. Moreover, in a systematic review including 15 RCTs, a reduction in cardiovascular risk was suggested when SFA is replaced by PUFAs, but not by MUFAs, carbohydrates, or protein [59].

2.3.4 Fruit and vegetables

Fruit and vegetables include several nutrients, dietary fibre, and phytochemicals. Furthermore, they generally have low energy density [60].

The association between a higher intake of fruit and vegetables and IHD risk has been investigated intensively [61–65]. In a recent meta-analysis of prospective cohort studies, fruit and vegetable consumption was associated with a 17% lower relative risk of CVD, found for the highest versus the lowest categories of fruit and vegetable intake [62]. In addition, a dose–response analysis showed that participants who ate 800 g/d of fruit and vegetables had the lowest risk of CVD [62]. This is supported by the results of another meta-analysis where the relative risk of stroke was 21% lower for the highest versus the lowest categories of fruit and vegetable intake, and the risk of stroke decreased 32% and 11% for every 200 g/d increase in consumption of fruit and vegetables, respectively [61]. This protective association was also revealed in a prospective study including two large cohorts [66]. However, no strong evidence was found for a

cardioprotective effect in individual RCTs with provision of fruit and vegetables, according to a meta-analysis that included 10 RCTs [65]. Despite this, the authors concluded that because the studies included in the meta-analysis were all short-term, and only limited amounts of fruit and vegetables were provided, an increased intake of fruit and vegetables should be advised because single interventions have showed beneficial effects on CVD risk factors. The authors further concluded that additional RCTs are needed to confirm this [65].

2.3.5 Whole grains and dietary fibre

A whole grain kernel includes all the edible parts of the kernel, including the bran, germ, and endosperm. Whole grains are the main source of dietary fibre and also contain vitamins, minerals, antioxidants, and phytochemicals [43, 67]. When the grain is refined, most of the bran and germ is removed, leaving the starchy endosperm to be further ground into white flour [67].

The standard definition of whole grain and whole grain products varies slightly from country to country. In Denmark, whole grains are defined as whole kernels and processed kernels, including the bran, germ, and endosperm in the same proportions as the whole kernel. Whole grain products are defined according to the amount of whole grain in the product. Flour and grain must be 100% whole grain to receive the Whole Grain label. For whole grain products that include other ingredients, the content of whole grain must be at least 35% for bread and 55% breakfast cereals, crisp bread, dry pasta, and noodles [68].

Extensive research has been done on the relationship between whole grain and fibre intake and CVDs, including IHD. In several recent meta-analyses and systematic reviews of both prospective cohort studies and RCTs, a high intake of whole grains was found to be associated with lower risk of IHD [67, 69–73].

2.4 Assessment of dietary intake

An essential component in dietary intake-related research is the availability of appropriate methods for dietary assessment. Many different dietary assessment methods exist, including 24-hour dietary recall, the diet record method, diet histories, and food frequency questionnaires (FFQ). The FFQ is the most commonly used method for measuring dietary exposures in epidemiologic studies [74].

The level of detailed information about food and nutrient intake varies considerably among dietary assessment methods and can play a role in the accuracy of estimating dietary intake. The method

used in any study should therefore be based on the foods or nutrients of interest and the capacity of the target population to provide the necessary details.

Diet histories and FFQs are retrospective recall methods designed to estimate the usual dietary intake over a longer period; 24-hour dietary recall involves detailed recollection of the dietary intake over the previous 24 hours. An FFQ is used to obtain the intake frequency of specific foods whereas diet histories are used to capture more detailed information about dietary intake [74].

The diet record method, on the other hand, involves prospective recording of dietary intake on typically 2 or more specific days. In addition, the dietary record method captures very detailed information about the foods and nutrients consumed over a specific stretch of time [74].

Increased use of modern technology to collect dietary information via computers, cameras, and mobile phones is a huge advance [75], as these new digital methods of dietary assessment make data collection much faster and cheaper [75]; however, these methods require individuals to have internet access and/or a mobile phone.

2.5 Dietary quality indices

Previously, nutritional research has been focused on single foods and nutrients and their related health effects. However, isolating nutrients and foods may not provide a realistic picture of what people eat in combination and the health effects thereof. The health effects may be the result of an additive or synergetic effect of many different components in the diet, which nutritional research focused on single food and nutrient components does not capture. Thus, increasing attention has recently been given to healthy dietary patterns and their relationship to disease outcomes such as IHD [8, 11, 76]. In addition, accumulated evidence supports an association between healthy dietary patterns and a decreased risk of CVDs [34].

Adherence to dietary patterns or dietary guidelines is often measured using indices or scores designed to capture the essential food or nutrient components of a relevant healthy diet or dietary guidelines [77]. A wide range of these dietary scores and quality indices have been developed [9, 11, 76, 78]. To calculate a single score for adherence, additional points are given for higher intake of foods and nutrients with health-promoting effects and lower intake of foods or nutrients with health-harming effects.

Some of the most commonly used scores and indices are the Mediterranean Diet Score, indicating compliance with the traditional dietary pattern followed by Mediterranean populations, and the American Healthy Eating Index (HEI), which assesses adherence to the Dietary Guidelines for Americans [79]. Both observational studies and RCTs have found a protective effect against development and mortality of CVD with greater adherence to a Mediterranean diet and the Dietary Guidelines for Americans [80–87].

2.6 Healthy dietary patterns or national dietary guidelines and IHD

In the two following sections, the most relevant studies with respect to background and discussion of this thesis research on healthy dietary patterns or national dietary guidelines and IHD are summarised. The studies have been selected based on literature searches conducted in PubMed and by reviewing the reference lists of the relevant literature.

In the first section (section 2.6.1) relevant Nordic and Danish observational studies investigating the association between healthy dietary pattern and national dietary guidelines, using dietary indices or scores and IHD are summarised.

In the second section (section 2.6.2) a summary of relevant dietary RCTs is given. The trials are divided according to study design on interventions with food provision and interventions without or with limited food provision.

2.6.1 Observational studies

In Europe, the Mediterranean diet has been widely promoted; however, adherence to the Mediterranean diet in countries outside the Mediterranean region remains low [88]. Differences in food culture, preferences, and availability of local sources might limit adherence to the Mediterranean diet outside that region. Therefore, several indices reflecting the food culture and dietary guidelines of Nordic countries, including Denmark, have been developed [89–93].

One example is the healthy Nordic food index, which is based on a 192-item FFQ including foods originating in the Nordic climate and reflecting the traditional Nordic diet. The included foods are fish, rye bread, oatmeal, cabbages, apples, pears, and root vegetables [93].

In two prospective studies by Gunge et al. [94] and Hansen et al. [95] based on the same cohort, the Danish Diet, Cancer and Health cohort including around 56,000 adult men and women, the association between adherence to a healthy Nordic diet and MI and stroke was investigated. When

comparing individuals with the highest healthy Nordic food index scores and those with the lowest index scores, lower risks of MI (men: hazard ratio (HR) 0.77, 95% CI 0.62 to 0.97; women: HR 0.55, 95% CI 0.37 to 0.82) and stroke (HR 0.86, 95% CI 0.76 to 0.98) were found [94, 95]. Another prospective cohort based on the Danish Diet, Cancer and Health cohort also found an inverse association between adherence to a healthy Nordic diet, assessed by the healthy Nordic food index, and type 2 diabetes (men: HR 0.62, 95% CI 0.53 to 0.71, women: HR 0.75, 95% CI 0.61 to 0.92), when individuals with the highest healthy Nordic food index scores were compared with those having the lowest index scores [96].

Despite this, results are inconsistent regarding a cardioprotective association of adherence to a healthy Nordic diet, as assessed by the healthy Nordic food index. In two prospective cohort studies by Roswall et al. based on the same cohort, the prospective Swedish Women´s Lifestyle and Health cohort including around 44,000 adult women, no overall association was found between the healthy Nordic food index and overall risk of CVD, IHD, and cardiovascular mortality [97, 98].

Examples of indices reflecting the Danish official dietary guidelines are the Dietary Quality Score (DQS) and Diet Quality Index (DQI), both developed to measure adherence to 2005 Danish dietary guidelines [90, 91].

The DQS was developed and validated in a cross-sectional study by Toft et al. [90], who also investigated the association between the DQS, as a measure of adherence to the 2005 Danish official dietary guidelines, and CVD risk factors [90]. The DQS was developed based on a 48-item FFQ, using a 3-point scoring system for each of four food groups: fish, fruits, vegetables, and fats, reflecting the Danish official dietary guidelines for 2005 [90]. The DQI was further validated against a 198-item FFQ. The study included 6542 healthy adult men and women. In the study, Toft et al. found that a higher DQS was inversely associated with total cholesterol, TAG, LDL cholesterol, homocysteine, and absolute risk of IHD (using the Copenhagen risk score) [90].

In addition, in a recently published prospective cohort study by Hansen et al. based on the Danish Diet, Cancer and Health cohort, adherence to the Danish official dietary guidelines 2013 as assessed by an updated DQI, called the Danish Dietary Guidelines Index Score, and risk of MI was investigated [95]. The updated DQI was based on a 192-item FFQ and included six foods and nutrients: whole grains, fish, fruit and vegetables, red and processed meats, and E% from saturated fat and added sugar. In all, 55,021 adult men and women were included. A higher

Danish Dietary Guidelines Index Score was associated with lower risk of MI among both men (HR 0.87, 95% CI 0.78 to 0.96) and women (HR 0.76, 95% CI 0.63 to 0.93) [99].

Although different in their description and composition, the dietary scores and indices reflect different healthy diets or national dietary guidelines all capture the essential elements of a healthy diet.

2.6.2 Randomised controlled trials

2.6.2.1 Interventions with food provision

The controlled feeding trial is one method used to test the effects of healthy dietary patterns or national dietary guidelines on IHD risk factors through RCTs. In a controlled feeding trial, participants are provided with all foods during the period of the intervention. The results of these studies allow for a more straightforward interpretation of dietary exposure.

A classic example of a controlled feeding trial is an RCT conducted by Appel et al. investigating the effect of the Dietary Approaches to Stop Hypertension (DASH) diet on blood pressure [100]. The study included 459 healthy adults, who were randomly assigned to either a control group or to a group following one of two DASH diets: a diet rich in fruits and vegetables or an extended diet rich in fruits, vegetables, and low-fat dairy products, and with reduced saturated and total fat [100, 101]. The participants were provided with all foods free of charge. Appel et al. found an improvement in blood pressure among participants following both types of DASH diet in comparison with the control group [100].

Nordic examples of more recently published controlled feeding trials are RCTs conducted by Adamsson et al. [102] and Poulsen et al. [103]. Adamsson et al. investigated the effects of a healthy Nordic diet on cardiovascular risk factors. The study included 88 hypercholesteraemic participants who were randomly assigned to an *ad libitum* Nordic diet or a control diet [102]. All meals and foods were provided free of charge to the group following the healthy Nordic diet; the control group did not receive any meals or foods. Compliance was assessed using a daily study checklist. Adamsson et al. found that participants receiving the healthy Nordic diet had improved lipid profiles and insulin resistance, measured by HOMA-IR and blood pressure, in comparison with participants who were assigned to the control diet [102].

In addition, Poulsen et al. investigated the health effects of the New Nordic Diet in an RCT [103]. The study included 147 adult participants with increased waist circumference who were randomised to receive either the New Nordic Diet or an average Danish diet (control). Cookbooks and all foods were provided free of charge to both groups through a shop model. A cookbook including recipes and menu plans was developed for the intervention group and a cookbook with recipes for traditional Danish dishes was developed for the control group. Dietary compliance was assessed using 3-day dietary records at three occasions during the intervention. Poulsen et al. found that in comparison with the group eating an average Danish diet, participants following the New Nordic Diet had improved lipid profiles, fasting glucose and CRP concentrations, blood pressure, and had reduced weight [103].

2.6.2.2 Interventions without or with limited food provision

Another approach to testing the effects of healthy dietary patterns or national dietary guidelines on IHD risk factors through dietary RCTs is by using a setting that is closer to real-life than in controlled feeding trials. Here, the study participant only receives dietary advice and/or some of the appropriate foods, with the aim to increase participants' motivation to adhere to the advice given. Otherwise, participants are completely empowered to modify their own dietary habits and patterns.

A recent RCT by Jenkin et al. investigated the effects of dietary advice with and without food provision on weight loss and cardiovascular risk factors [104]. The study included 919 healthy overweight adult men and women. The study participants were randomised to either a control group who received the Health Canada´s Food Guide and nothing else, or one of three intervention arms. In addition to the Health Canada's Food Guide, received by all three intervention arms, the first intervention arm received dietary advice based on the DASH diet and the Portfolio diet (a dietary approach to lowering cholesterol); the second intervention arm was provided foods reflecting the same dietary advice each week (without receiving dietary advice); and the third intervention arm was provided both foods and dietary advice. Additional interventions included 20–30 minutes of telephone interviews conducted weekly during the first month and monthly during the last 5 months of the intervention. The duration of the intervention was 6 months, with a 12-month follow-up. Dietary assessment was done via a validated FFQ, which was expanded to capture whole grains and viscous fibre. The retention increased with the provision of foods. Apart from an increase in whole grain intake in all three groups, an increase in the intake of other recommended foods was found only in the groups receiving foods, compared with the control group. No difference in CVD risk

factors was seen from baseline to 6 months or from baseline to 18 months in any of the three intervention groups, in comparison with the control group [104].

Another 12-week RCT, the CRESSIDA study by Reidlinger et al. [105], including 162 healthy middle-aged and older men and women, investigated the effects of adherence to the United Kingdom (UK) dietary guidelines on selected CVD risk factors [105]. The study participants were randomised to a group following UK dietary guidelines or to a control group instructed to eat a traditional British diet. The UK dietary guidelines included recommendations for reduced intake of SFA, added sugars, and sodium and increased intake of oily fish, fruit and vegetables, and whole grains. Adherence was assessed through dietary interviews conducted by a dietitian, twice face-to-face (baseline and week 4) and twice by e-mail or phone calls (weeks 6 and 8). Food provision to participants following UK dietary guidelines included low-saturated/*trans*-fat margarine and liquid vegetable oil (high-oleic sunflower oil). Food provision to participants in the control diet group included high-saturated/*trans*-fat butter-based spread and liquid hydrogenated vegetable oil (olein). All participants were further provided with other foods such as whole grain breakfast cereal, brown rice, snacks (e.g., nuts and chocolate bars), and tinned fish (tuna, mackerel). Adherence to the dietary advice was assessed through 4-day diet records and biomarkers of intake. The overall dietary changes in the group following UK dietary guidelines, compared with the control group, was an increased intake of whole grains, dietary fibre, and E% intake from protein; a decreased intake of sodium and E% intake from total fat, SFA, and *trans*-fatty acids; and an increased E% intake from MUFAs and PUFAs. In addition, Reidlinger et al. found a decrease in systolic blood pressure, serum lipids, CRP, and waist circumference in the group following UK dietary guidelines in comparison with the control group [105].

In a RCT, the SYSDIET study, Uusitupa et al. [106] examined the effects of an isocaloric (to avoid weight loss) healthy Nordic diet on cardiometabolic risk factors [106]. The 18- to 24-week study included 166 adult men and women with features of metabolic syndrome, who were randomly assigned to eat a healthy Nordic diet or a control diet (average Nordic diet). Key food items were provided free of charge in both groups. The healthy Nordic diet group received key products such as whole grain products, berry products, dietary fats including rapeseed oil and spreads based on vegetable oils, and fish or covered expenses for fish consumed. The control group received low-fibre cereal products and dairy fat-based spread, e.g., butter. A dietitian introduced the diets to the groups at baseline and compliance was measured repeatedly thereafter using 4-day food records; in

addition, serum phospholipids were analysed for fatty acid composition. Overall dietary changes between the groups favoured the group that adhered to the healthy Nordic diet, with changes in carbohydrates, protein, total fat, SFA, PUFAs, dietary fibre, and salt and sodium between the groups. Uusitupa et al. found a decrease in non-HDL cholesterol (total cholesterol – HDL cholesterol), LDL-to-HDL cholesterol ratio, and apolipoprotein B/apolipoprotein A1 ratio in the healthy Nordic diet group, compared with the control group. No changes in insulin sensitivity or glucose tolerance was found [106].

The investigated endpoints of the above-mentioned studies are all intermediate risk factors for IHD and CVD. Long-term dietary RCTs investigating the effects of healthy dietary patterns or national dietary guidelines on incidence of diseases such as IHD as the endpoint are rare and are often not feasible. In such long-term trials, compliance with the intervention diets will likely decline during the intervention period, and they require a larger study population and longer duration than intermediate-term trials; this results in prohibitive costs of conducting long-term trials.

However, one example of such a long-term dietary RCT is the PREDIMED study [107]. In the PREDIMED study, Estruch et al. investigated the effects of two different Mediterranean diets, compared with a low-fat diet, on the incidence of major cardiovascular events [107]. In all, 7447 middle-aged men and women with high cardiovascular risk were included in the study, with a median follow-up of 4.8 years. Participants were randomised to a Mediterranean diet supplemented with mixed nuts, a Mediterranean diet supplemented with extra-virgin olive oil, or a control group advised to follow a low-fat diet. Mixed nuts and extra-virgin olive oil were provided free of charge to the two intervention groups; the control group received small non-food gifts. Additional intervention in the two Mediterranean diet groups included dietary training with a dietitian in individual and group sessions at baseline and quarterly thereafter. The control group also received dietary training by a dietician at baseline; thereafter, participants received leaflets explaining the low-fat diet on a yearly basis for the first 3 years of the intervention. Dietary compliance was measured using a 137-item FFQ and biomarkers were assessed to determine intake of extra-virgin olive oil (urinary hydroxytyrosol levels) and mixed nuts (plasma alpha-linolenic acids). At the end of the intervention, the largest dietary changes were differences in the composition of fat subtypes, fish, and legumes consumed. Estruch et al. found a 30% and 28% lower risk of major cardiovascular risk in the groups assigned to the Mediterranean diets with extra-virgin olive oil and nixed nuts, respectively, compared with the low-fat diet group [107].

To sum up, of the presented dietary RCTs with or without limited provision of key food products, and conducted in a real-life setting, both the study by Reidlinger et al. [105] and that by Uusitupa et al. [106] enrolled a control group that was provided with foods high in SFA. In the PREDIMED study by Estruch et al., the control group did not receive any SFA-rich foods but instead were advised to eat low-fat products [107]. The control group in the study by Jenkins et al. only received a leaflet with dietary guidelines, which was also provided to the three other intervention arms [104]. In addition, the intervention groups in the studies by Reidlinger et al. [105], Uusitupa et al. [106], and Estruch et al. [107] were all provided food products high in unsaturated fat.

There are differences regarding the dietary assessment methods followed in these studies. In the study by Jenkins et al. [104] and in the PREDIMED study by Estruch et al. [107], an FFQ was used to assess adherence. In contrast, both the CRESSIDA study by Reidlinger et al. [105] and the SYSDIET study by Uusitupa et al. [106] used 4-day dietary records. In addition, the PREDIMED, CRESSIDA, and SYSDIET studies all included biomarkers of intake [105–107]. Finally, the intensity of the intervention in the presented RCTs with or without limited food provision, such as the extent of dietary counselling provided, also differed between these studies.

3 Methods

3.1 Study design

From March 2014 to May 2015, a 6-month single-blinded parallel RCT with a 6-month follow-up was conducted in a real-life setting and included adult participants with a minimum of one self-reported risk factor of IHD. At baseline participants were assigned to either a control group advised to follow their habitual diet or to one of two intervention groups receiving either targeted substitution dietary guidelines or the Danish official dietary guidelines.

Short- and long-term effects of the guidelines were defined as the changes in diet and IHD risk factors from baseline to 6 months (end of the intervention) and from baseline to 12 months (follow-up), respectively.

This study was part of the research project ´Diet and Prevention of Ischemic Heart Disease – a Translational Approach´ (DIPI) (www.DIPI.dk). The study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by The Capital Region of Denmark Ethics Committee (Journal no. H-1-2013-110) and by the Danish Data Protection Agency (Journal no. 2013-54-0571). Written informed consent was obtained from all study participants, and they received a small remuneration of around 34 GBP for their participation in the study. The study was registered at ClinicalTrials.gov (registry name "Diet and Prevention of Ischemic Heart Disease: a Translational Approach (DIPI)", ID no. NCT02062424).

3.2 Study participants

Potential participants were identified using a unique personal identification number assigned to all Danish citizens in the Civil Registration System [108]. In total, 5000 men and women born during 1949–1984 and living in a defined area of Greater Copenhagen were invited by letter to participate in the study. The number of invited participants was based on previous experience of a low response rate when recruiting participants for RCTs. Overall, 334 people responded to the invitation and were screened using a self-administered questionnaire that included questions on the inclusion and exclusion criteria (**Figure 2**).

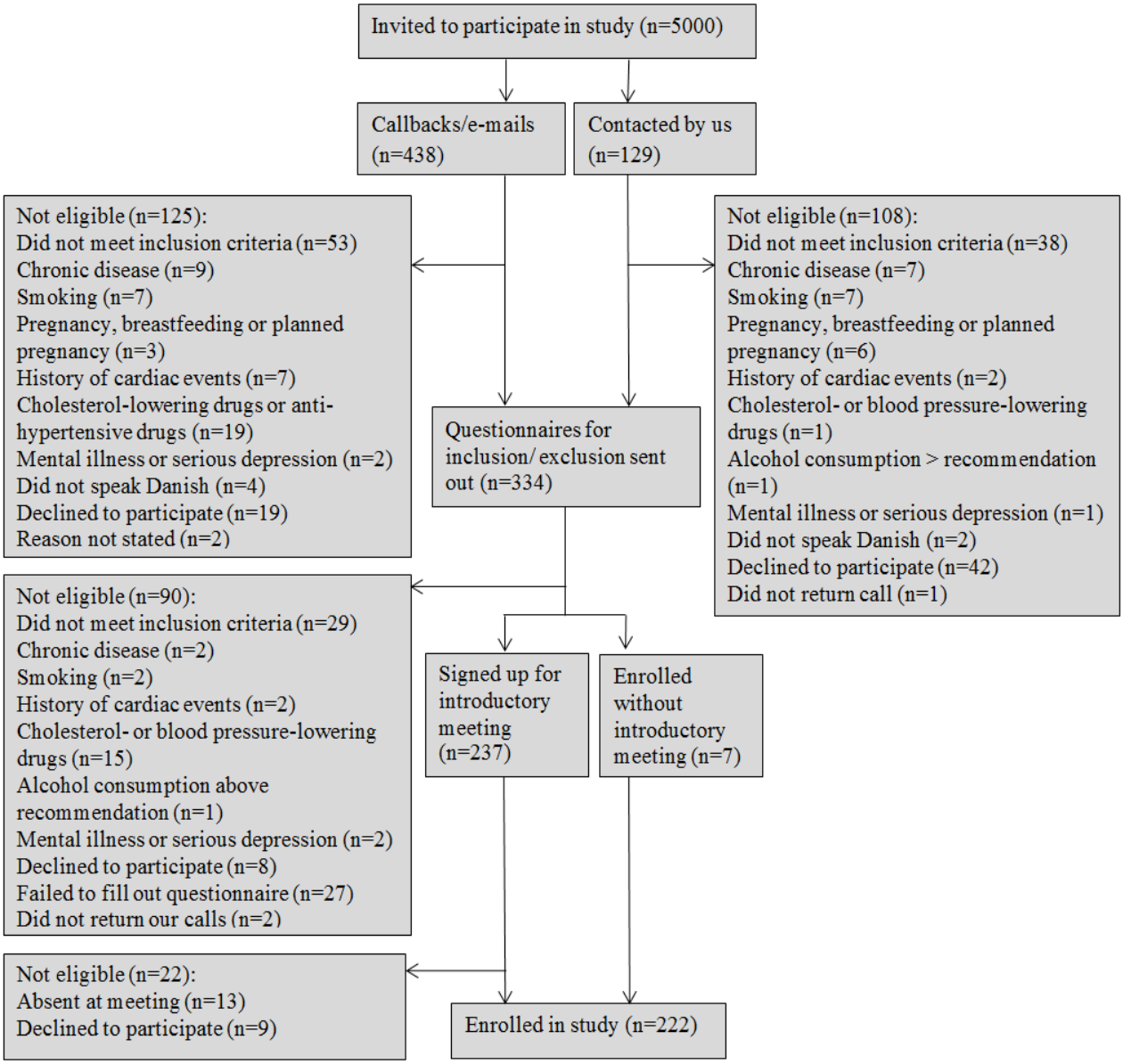


Figure 2 Flow chart of participant recruitment

Potential participants were asked to measure and report their height in metres, weight in kilograms (kg), their waist circumference at a height 2 cm above the umbilicus, and whether they were physically active for more than 15 min/wk. Furthermore, the self-administered questionnaire included questions on the exclusion criteria. Eligible participants were invited to an informational meeting, which included an introduction to the web-based dietary assessment software.

3.2.1 Inclusion and exclusion criteria

The inclusion criteria were age between 30 and 65 years, and a minimum of one self-reported risk factor for IHD: overweight or obesity (BMI ≥ 25), waist circumference ≥ 80 cm for women and ≥ 94 cm for men, and/or physical inactivity defined as being moderately physically active during leisure time for 15 minutes or less per week.

Exclusion criteria were current smoking, pregnancy or plans to become pregnant within the next 12 months, breastfeeding, history of CVD, type 2 diabetes, chronic diseases/disorders that could affect the results of the study (chronic diseases reported by participants were evaluated by the physician in charge), drug abuse within the last 12 months, regular alcohol consumption > 21 units/week for men or > 14 units/week for women[1], allergies or intolerance of the food groups included in the dietary guidelines, consumption of dietary supplements with high doses of nutrients that could have a potential effect on IHD risk factors (e.g., fish oils), and/or no access to a computer or the internet.

3.3 Randomisation and intervention

A schematic overview of the study design is presented in **Figure 3.** After the baseline examination, participants were randomly assigned to one of the three study groups using a computer randomisation plan (www.randomization.com) for men and women separately, to ensure that the randomisation was balanced by sex.

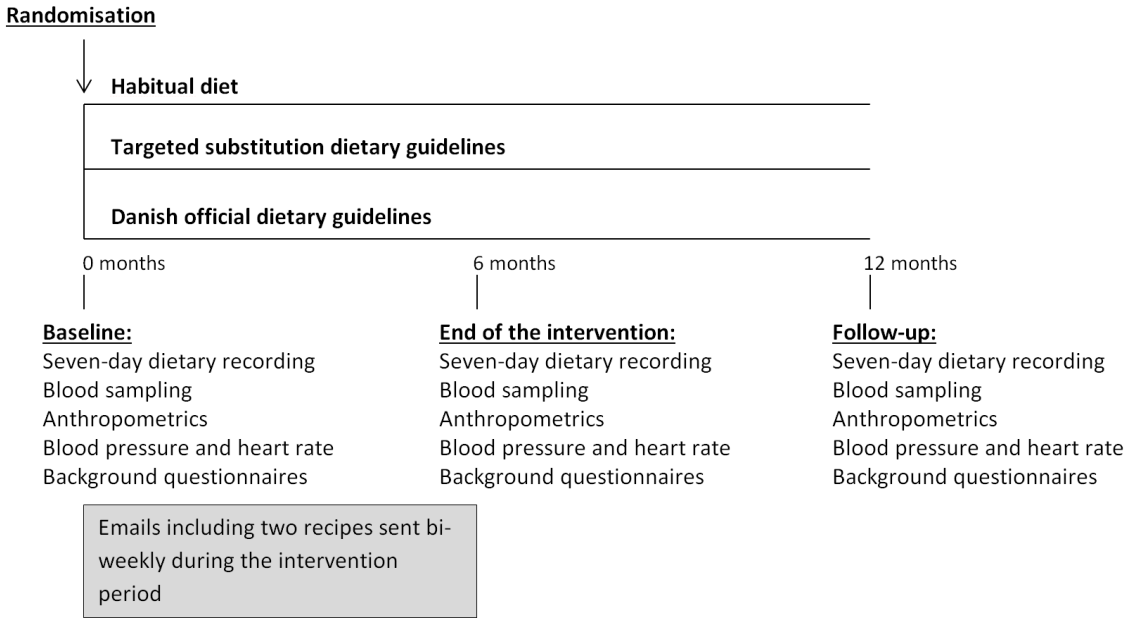


Figure 3 Schematic overview of the study design.

[1] One unit (DK) is 12 g of pure alcohol.

The guidelines given to participants in the targeted substitution dietary guidelines group focused on 5 of the 10 Danish official dietary guidelines related to foods or nutrients (**Table 3**) where the scientific evidence for a relationship between a dietary factor and an IHD outcome was found to be convincing or probable [5]. Moreover, exact substitutions were specified.

The guidelines given to participants in the Danish official dietary guidelines group included all 10 official guidelines on foods, beverages, and physical activity (**Table 3**). The official dietary guidelines were updated based on a systematic literature update of the scientific literature on the associations between food intake and physical activity and different diet-related diseases, on knowledge of Danish food habits and the Nordic Nutrition Recommendations 2012 [5].

Table 3 Targeted substitution dietary guidelines and Danish official dietary guidelines.

Substitution dietary guidelines	Official dietary guidelines
	Eat a variety of foods, but not too much, and be physically active
Eat fruit instead of candy and cake	Eat fruits and many vegetables
Eat coarse vegetables instead of fine vegetables†	
Eat fish instead of red meat	Eat more fish
Eat whole grain products instead of products with no whole grains	Choose whole grains
	Choose lean meats and cold meats
	Choose low-fat dairy products
Eat unsaturated fat instead of saturated fat	Eat less saturated fat
	Eat foods with less salt
	Eat less sugar
	Drink water

† Vegetables are classified by type (e.g., all types of cabbage, root vegetables, and onions are classified as coarse vegetables and all vegetables with a high water content, like tomatoes and salad greens, are classified as fine vegetables).

The two study groups assigned to receive either the targeted substitution dietary guidelines or the Danish official dietary guidelines were provided information about the guidelines via a letter, which included a leaflet containing the respective guidelines, and also via a website (www.dipi.food.dtu.dk). The participants were given a personal password to the website where they could find leaflets and recipes. The leaflets and recipes for the targeted substitution dietary guidelines and the Danish official dietary guidelines were identical in design, structure, and number; only the content varied according to the dietary guidelines each participant was randomly assigned to follow. All recipes given to the intervention groups were developed by the Danish Veterinary and Food Administration and were Keyhole nutrition labelled [40]. To increase the participants' motivation and compliance with the respective dietary guidelines, an e-mail with two

new recipes per intervention group was sent out to participants biweekly during the intervention period. In the group following the targeted substitution dietary guidelines, only recipes for fish dishes were sent. To increase motivation and compliance in the habitual diet group, participants were also sent an e-mail every second week, including a 'thank you for still participating' greeting.

At the end of the intervention after 6 months, participants were told that the intervention portion of the study was finished but that they would be re-invited to a follow-up examination in another 6 months (**Figure 3**).

3.4 Measures

3.4.1 Dietary assessment

Study participants recorded their dietary intake for 7 consecutive days, using a self-administered web-based dietary assessment software [109]. The software is based on a validated diet recording method including a 7-day food record, which has been used for the past two decades in the Danish National Survey of Dietary Habits and Physical Activity [37, 110]. The web-based dietary assessment software was originally developed and validated for children aged 8–11 years and has been customised to fit the adult participants in the DIPI study [109, 111, 112]. A user manual for the software was given to study participants at baseline. At least 4 days of food reporting had to be completed for participants to be included in the analysis [110].

The dietary assessment software was structured according to a typical Danish meal pattern covering breakfast, lunch, dinner and three in-between meals. The participants could estimate the amount consumed by selecting the closest portion size from among four different digital images in an 80-photograph series. Reminders for frequently overlooked foods (e.g., spreads, sugar, sauces, dressings, snacks, candy, and beverages) were included. Furthermore, participants reported the intake of nutritional supplements and whether each day represented the usual or an unusual intake, and included reasons for unusual intake, such as illness. If a participant failed to report on one day, they were sent a reminder e-mail the following day [109].

Intakes of food items, energy, and nutrients were calculated for each study participant as an average of 7 days using the software General Intake Estimation System (GIES) version 1.000.i6 and the Danish Food Composition Databank version 7.0 (National Food Institute, Technical University of Denmark).

3.4.1.1 Under- and over-reported energy intake

Under- and over-reported energy intake was defined as a ratio of reported mean energy intake to basal metabolic rate and classified using cut-offs suggested by Black [113, 114]. Under-reported energy intake was defined as; ratio of reported mean energy intake to basal metabolic rate ≤ 1.05 and over-reported energy intake was defined as; ratio of reported mean energy intake to basal metabolic rate ≥ 2.28, using a physical activity level 1.55 (data not shown). Basal metabolic rate was calculated using the equations by Henry [115].

3.4.1.2 Calculation of DQI

Adherence to the Danish official dietary guidelines was evaluated based on a previous DQI, updated to the current Danish official dietary guidelines [91, 116], which include intake of whole grains (min 75 g/10 MJ/d), intake of fish (min 350 g/wk), intake of fruits and vegetables (min 600 g/10MJ/d), energy from saturated fat (max 10 E%), and energy from added sugar (max 10 E%). The DQI was based on intake adjusted to 10 MJ as this is the unit used in the dietary guidelines [5].

A DQI score for each study participant was calculated as the ratio of the actual intake and the recommended intake of each of the five guidelines included in the index (adapted from Knudsen et al. [91]). For example, if a study participant had an intake of 60 g/10 MJ/d of whole grains, the score was 60/75 = 0.8. For the included guidelines with an upper limit for a recommended intake, the DQI was calculated as 1−[(intake−recommended)/recommended]; thus, for a study participant with an intake of 13% E from added sugar, the DQI was calculated as 1−[(13−10)/10] = 0.7.

In contrast to the original DQI, we did not have a maximum score for individuals with an intake exceeding the cut-off values [91]. The total score was calculated as the sum of the five scores, with a higher score indicating a greater degree of adherence to the Danish official dietary guidelines.

3.4.2 Blood samples (paper III)

Fasting blood samples from venepuncture were analysed for concentrations of TAG, total cholesterol, HDL cholesterol, high-sensitivity C-reactive protein (hsCRP), glucose, HbA_{1c}, and insulin. The blood samples were collected and handled according to hospital routines. TAG, total cholesterol, HDL cholesterol, and glucose were measured in plasma by reflection spectroscopy, with peaks at 540 nm; hsCRP was measured in the same way, with peaks at 660 nm (VITROS 5,1 FS; Ortho Clinical Diagnostics, Bridgend Pencoed, United Kingdom). HbA_{1c} was measured in plasma using high-performance liquid chromatography (D-100; Bio-Rad, Copenhagen, Denmark).

Fasting plasma insulin was measured using the sandwich enzyme-linked immunosorbent assay (ELISA) analysis principle (ADVIA Centaur XP; Siemens, Ballerup, Denmark). Within-run variation (coefficient of variation; CV%) for the biochemical measurements was 0.7%–11%. VLDL cholesterol was calculated from TAG using the following equation: plasma VLDL cholesterol = plasma TAG × 0.45. LDL cholesterol was calculated using the Friedewald equation [117].

HOMA-IR was used to estimate insulin resistance and was calculated using the following formula: HOMA-IR = [glucose (nmol/L) × insulin (mU/mL)/22.5], using fasting values [22].

3.4.2.1 Plasma alkylresorcinol concentrations (paper II)

Total alkylresorcinol concentrations were extracted and purified from plasma samples and analysed using gas chromatography–mass spectrometry (Finnigan TRACE GC Ultra Gas Chromatograph coupled to a Finnigan TRACE DSQ II mass detector; Thermo Fisher Scientific, Waltham, MA, USA), according to a method described elsewhere in detail [118].

3.4.3 Blood pressure and heart rate (papers I to III)

Blood pressure and heart rate were measured in duplicate on the left arm, with the participant seated and after a 5-minute rest, using an electric sphygmomanometer according to standard procedures. Participants were asked to empty their bladder before measurement and were not allowed to converse during the measurements or to have their legs crossed. If the diastolic blood pressure differed more than 5 mmHg between measurements, further readings were taken until diastolic blood pressure differed ≤ 5 mmHg between at least two consecutive measurements. Average values of the two blood pressure and heart rate measurements were calculated.

3.4.4 Anthropometric measurements (papers I to III)

Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer (seca, Hamburg, Germany). Fasting body weight in kg and abdominal obesity were registered on a body composition analyser (BC-418MA; Tanita, Tokyo, Japan). Waist- and hip circumference were measured twice using an anthropometric tape (seca 201) and the average was reported. BMI was defined as weight in kg divided by squared height in meters (kg/m^2).

3.4.5 Background questionnaires (papers I to III)

Lifestyle questionnaires were used to obtain information about the participant's education level (primary school/high school, associate degree, undergraduate degree, graduate degree) and level of

physical activity during leisure time (extremely active, moderately active, sedentary, or inactive). The question addressing physical activity level queried participants' physical activity during leisure time in the previous 6 months and was based on the Danish National Health Profile questionnaire [119].

3.5 Statistical method

For a parallel design, statistical power calculations based on evidence from previous similar studies [120–122] were used to estimate that 62 participants in each intervention arm was sufficient to detect a difference of 0.25 mmol/L LDL cholesterol (SD, 0.49) ($\alpha = 0.05$, $\beta = 0.8$). To allow for a dropout rate of 20%, the number of participants was set to a total of 225. Using a paired *t*-test, self-reported weight (kg), waist circumference, and BMI from the screening self-administered questionnaire were compared with weight, waist circumference, and BMI measured at baseline. Baseline characteristics and dietary intake of the participants were summarized for men and women using medians and 80% central range for continuous variables and proportions for categorical variables.

Baseline differences in dietary intake endpoints and IHD risk factors between the randomised groups were evaluated using analysis of variance (ANOVA) for continuous variables and the Kruskal–Wallis test for categorical variables (data not shown).

For papers I and II, sensitivity analyses excluding under- and over-reporters were conducted to investigate the impact of under- and over-reported energy intake on the results of these papers.

All statistical analyses in the three papers were carried out using RStudio Version 0.99.441 (RStudio, Inc., Boston, MA, USA). Statistical significance was set at $p < 0.05$.

3.5.1 Paper I

Linear regression analyses were applied to evaluate the association between DQI and cardiometabolic risk factors. Three models were used: a simple model adjusted for sex and age (< 50 or ≥ 50 years) (model 1a); a multivariate model further adjusted for education level (primary school/high school, associate degree, undergraduate or graduate degree) and physical activity during leisure time (extremely active, moderately active, sedentary, or inactive) (model 1b); and a final multivariate model adjusted as in model 1b plus BMI (model 2). Furthermore, sensitivity analysis excluding under- and over-reporters was done to investigate the impact of under- and over-reporters on the associations between DQI and cardiometabolic risk factors.

All models were tested for statistically significant interactions between DQI and sex and DQI and age. These interactions were tested to investigate if the associations were different for men and women and for participants aged < 50 or ≥ 50 years. If an interaction was significant, the DQI estimates for men and women and DQI estimates by age were given.

To check the model assumptions, the standardized residuals of the final models were examined for normality, variance homogeneity, and linearity. BMI, waist-to-hip ratio, hsCRP, VLDL cholesterol, TAG, glucose, insulin, and HOMA-IR were all logarithm 10 transformed to normalize the distribution and to improve variance homogeneity. For the above-mentioned variables that were log transformed, the estimates and 95% confidence intervals (95% CI) are presented as percent difference.

3.5.2 Papers II and III

Two multiple linear regression models were applied to evaluate changes from baseline to 6 or 12 months in dietary intake endpoints, DQI score, and IHD risk factors in the targeted substitution dietary guidelines group and the official dietary guidelines group, compared with the habitual diet group. Model 1 was adjusted for baseline value of the outcome variable, and model 2 was further adjusted for sex, age group (< 50 and ≥ 50 years), and BMI group (BMI 18.5–25 as normal weight, > 25–30 overweight, and > 30 obese). In Model 2 interactions between the intervention group and sex, the intervention group and age group, and the intervention group and BMI group were additionally tested for statistically significance.

These interactions were tested to investigate whether the intervention had different effects for men and women; for participants above or below age 50 years; or for normal weight, overweight, or obese study participants. If an interaction was statistically significant, separate results were provided according to the level of the effect modifier.

To check the model assumptions, the standardised residuals of the final models were examined for normality, variance homogeneity, and linearity.

3.5.2.1 Statistical method for analysis with alkylresorcinols (paper II)

Whole grain intake and total alkylresorcinol concentration in plasma were grouped into quartiles, and cross-tabulation for total whole grain intake and alkylresorcinol concentrations in plasma was done to examine the agreement between quartiles using the baseline data. To validate the whole

grain intake estimated using the web-based dietary assessment, a simple linear regression model was used to test the association between whole grain intake and total alkylresorcinol concentrations in plasma at baseline. To normalise the distribution of the residuals and to improve variance homogeneity, alkylresorcinol concentrations and whole grain intake were logarithm 2 transformed.

3.5.3 Statistical analysis of within group differences

Paired *t*-tests were applied to investigate changes in dietary intake endpoints and IHD risk factors in the randomised groups, from baseline to 6 months and from baseline to 12 months.

4 Results

A summary of the results of the three papers (I to III), as well as data not included in the papers, is presented in this section (**Tables 4–10**). Details of the results for all statistical models in the three papers are found in **Appendix A**.

4.1 Baseline characteristics of the study participants

A total of 222 participants met the inclusion and exclusion criteria and were enrolled in the study, accounting for 67% of the initially screened potential study participants. Participants' self-reported weight and waist circumference were significantly higher ($p < 0.05$) than the measured weight and waist circumference at baseline; however, no difference in BMI was found. In all, 17% of women and 7% of men did not meet the inclusion criteria when measured at baseline (**Table 4**).

Table 4 Weight (kg), body mass index (kg/m^2), and waist circumference, self-reported and measured at baseline.

	Self-reported	Measured	
	Mean (SD)	Mean (SD)	p-value†
Weight (kg)	84 (16)	83 (16)	0.042
BMI (kg/m^2)	27 (4)	27 (4)	0.193
Waist circumference (cm)	94 (12)	93 (12)	0.034

†Paired *t*-test

A full baseline examination was missing for three participants; these individuals were therefore excluded from the analysis (see flow chart in **Figure 4**). Baseline characteristics of the remaining 219 study participants are presented in **Table 5,** by sex (for baseline characteristics of the study participants according to randomised intervention group, see papers II and III**)**. Of the 219 study participants, 90 were men and 129 were women. The median (p10–p90) age of the study participants were 51 (37–61) years. Most study participants were overweight or obese according to BMI. No differences were found in either of the dietary intake endpoints or the cardiometabolic risk factors between the interventions groups at baseline (data not shown).

Table 5 Baseline characteristics of the study participants according to sex; medians (p10-p90) or percentages (n)

Participant characteristics	All (n=219)	Men (n=90)	Women (n=129)
Age (years)	51 (37-61)	51 (36-61)	51 (37-61)
Metabolic markers			
Weight (kg)	83 (65-104)	88 (79-117.5)	75 (62.3-92.8)
BMI †	27 (23-34)	27 (24-34)	26 (23-33)
Weight status †:			
Normal weight, *%* (n)	27 (60)	16 (14)	36 (46)
Overweight, *%* (n)	53 (116)	66 (60)	43 (56)
Obese, *%* (n)	20 (43)	18 (16)	21 (27)
Waist circumference (cm)	92 (80-105)	97 (91-118)	87 (77-100)
Hip circumference (cm)	107 (99-120)	107 (102-119)	108 (99-120)
Systolic BP (mm Hg) ‡	130 (110-154)	135 (119-164)	125 (108-149)
Diastolic BP (mm Hg) ‡	80 (69-94)	82 (72-98)	78 (68-92)
hsCRP (mg/L) ¨	1.4 (0.2-6.3)	1.0 (0.2-5.4)	1.9 (0.2-8.3)
Lipid biomarkers ^			
Total cholesterol (mmol/L)	5.3 (4.1-6.8)	5.5 (4.4-6.9)	5.2 (4.0-4.5)
LDL cholesterol (mmol/L)	3.2 (2.2-4.5)	3.5 (2.5-4.7)	3.1 (2.1-4.5)
HDL cholesterol (mmol/L)	1.4 (1.0-3.9)	1.2 (0.9-1.9)	1.6 (1.1-2.3)
LDL/HDL-ratio	2.3 (4.1-6.8)	2.8 (1.7-4.3)	2.0 (1.2-3.0)
VLDL cholesterol (mmol/L)	0.5 (0.3-1.0)	0.6 (0.3-1.2)	0.4 (0.3-0.8)
TAG (mmol/L)	1.1 (0.6-2.3)	1.3 (0.7-2.6)	0.9 (0.6-1.8)
Glycaemic biomarkers §			
Glucose (mmol/L)	5.5 (5.0-6.3)	5.6 (5.1-6.4)	5.4 (4.9-6.1)
HbA_{1c} (%)	5.0 (4.5-5.5)	5.0 (4.6-5.5)	5.0 (4.5-5.5)
Insulin (pmol/L)	58 (30-100)	59 (31-127)	58 (30-94)
HOMA-IR	2.0 (1.0-3.8)	2.2 (1.0-4.6)	2.0 (1.0-3.6)
Educational level			
Primary school or high school, *%* (n)	25 (55)	29 (26)	23 (30)
Associate degree, *%* (n)	8 (18)	6 (5)	10 (13)
Undergraduate school, *%* (n)	40 (87)	31 (28)	46 (59)
Graduate school, *%* (n)	27 (58)	34 (31)	21 (27)

Abbreviations: BMI, Body Mass Index; BP, blood pressure; hsCRP, high-sensitivity C-reactive protein; LDL, low density lipoprotein-cholesterol; HDL, high density lipoprotein-cholesterol; VLDL, very low density lipoprotein-cholesterol; TAG, triglycerides; HbA_{1c}, haemoglobin A_{1c}; HOMA-IR, homeostatic model of insulin resistance

† BMI is calculated as weight in kilograms divided by the square of height in meters (kg/m^2). 18.5-25 = Normal weight, 25-30 = Overweight, >30 = Obese

‡ All; n=216, men; n=89, women; n=127, after exclusion of those using BP-lowing medication

¨ All; n=204, men; n= 87, women; n=117, due to lack in biochemical analyses of hsCRP

^ All; n=214, men; n=85, after exclusion of those using cholesterol lowing medication

§ All; n=218, women; n=128, as it was not possible to draw enough blood to the glycaemic biomarker analysis from one of the female study participants.

4.2 Completers

Altogether, 203 study participants completed the examination at 6 months and 199 also completed the dietary recording at 6 months, yielding compliance rates of 93% and 90%, respectively. At 12 months, 196 study participants completed the examination and 186 participants completed the dietary recording. The dropout proportion was not higher than the expected approximately 20% in each group, and did not differ between the groups. Reasons for non-completers are given in the flow chart **(Figure 4)**.

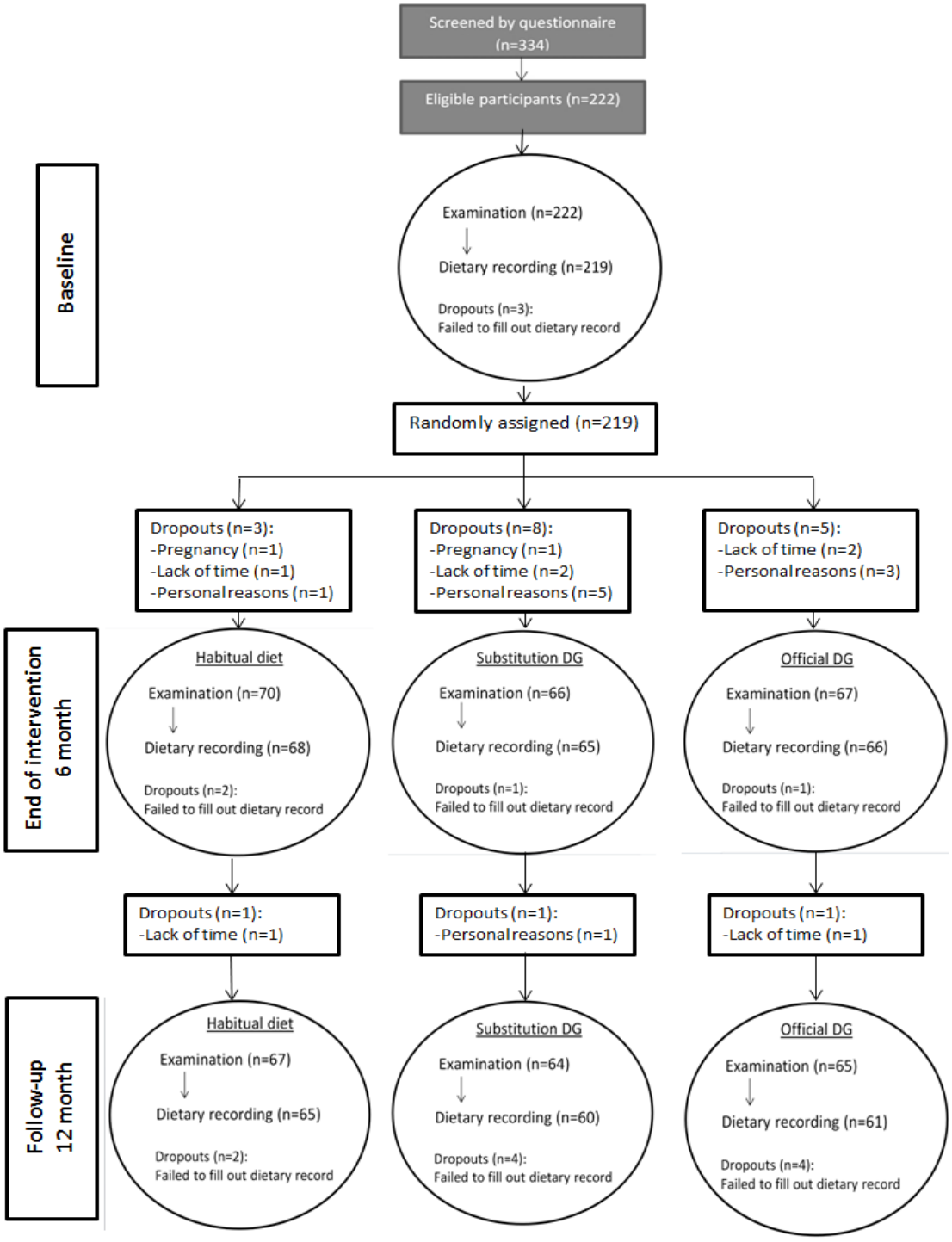


Figure 4 Flow chart of the study

4.3 Results of paper I

The results of paper I are based on the baseline data. **Table 6** presents the median (p10–p90) DQI score and individual DQI scores for the five included foods and nutrients, energy (MJ/d), diet composition (g/10 MJ/d), and energy contribution (E%) of macronutrients and dietary fibre (g/MJ) for study participants.

The median (p10–p90) DQI for the entire study population was 4.5 (3.0–6.5). The median DQI score was higher for women than for men. In **Table 1** of **Appendix B**, supplementary material is presented on the dietary intake endpoints of the study population, according to tertiles of the DQI score.

Table 6 Overall Diet Quality Index (DQI) score and individual DQI scores for the five included foods and nutrients, energy (MJ/d), diet composition (g/10 MJ/d), and energy contribution (E%) of macronutrients and dietary fibre (g/MJ) for study participants; medians (p10-p90).

Participant	Recommended†	All (n=219)	Men (n=90)	Women (n=129)	DQI score
Overall DQI		4.5 (3.0, 6.5)	4.2 (2.7, 6.2)	4.6 (3.0, 6.6)	
Energy MJ/d		8.6 (5.8, 12.4)	10.1 (7.2, 13.7)	7.8 (5.2, 10.5)	
Diet composition (g/10MJ/d):					
Bread and cereals‡		218 (141, 301)	215 (149, 291)	220 (136, 307)	
Whole grains	75 g/d	61 (31, 111)	56 (30, 117)	66 (34, 108)	0.8 (0.4, 1.5)
Meat‡	< 70 g/d	139 (59, 249)	166 (85, 282)	124 (45, 224)	
Fish‡ ¨	50 g/d	46 (5, 111)	41 (5, 109)	48 (5, 125)	0.9 (0.1, 2.2)
Poultry‡^		32 (0, 102)	31 (0 , 103)	32 (0, 99)	
Fruit and vegetables‡	600 g /d§	388 (177, 690)	324 (144, 508)	452 (243, 749)	0.6 (0.3, 1.2)
Fruit‡		143 (43, 331)	100 (23, 245)	180 (64, 362)	
Vegetables‡		220 (110, 425)	194 (84, 348)	237 (132, 469)	
Vegetables, coarse ‖		97 (29, 208)	83 (23, 191)	104 (39, 215)	
Vegetables, fine ‖		114 (49, 228)	95 (37, 167)	122 (68, 255)	
Potatoes‡		57 (7, 138)	73 (15, 152)	47 (2, 126)	
Milk‡		243 (68, 513)	213 (68, 484)	272 (70, 531)	
Cheese‡	Choose low fat	45 (11, 129)	36 (5, 97)	50 (16, 156)	
Edible fats		31 (18, 46)	30 (16, 48)	31 (18, 45)	
Sugar and candy	Reduce intake	35 (10, 77)	31 (10, 76)	38 (14, 77)	
Energy distribution					
Protein, E%	10-20	17 (14, 22)	17 (14, 21)	17 (14, 22)	
Fat, E%	25-40	35 (29, 42)	35 (28, 40)	36 (29, 43)	
SFA, E%	< 10	13 (11, 17)	13 (10, 17)	13 (11, 16)	0.7 (0.4, 0.9)
MUFA, E%	10-20	13 (10, 17)	13 (10, 16)	14 (11, 18)	
PUFA, E%	5-10	5 (4, 7)	5 (4, 6)	5 (4, 7)	
Carbohydrate E%	45-60	44 (35, 53)	43 (34, 53)	45 (35, 53)	
Added sugar E%	< 10	7 (2,13)	7 (2, 15)	7 (3, 13)	1.3 (0.7, 1.8)
Dietary fibre, g/MJ	>3 g/MJ	2 (2,3)	2 (2, 3)	3 (2, 4)	
Alcohol, E%¶	< 5	5 (1,14)	7 (1, 16)	4 (0, 10)	

Abbreviations: g, gram; MJ, mega joule; SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

† recommended according to the official Danish food based dietary guidelines [5] and the Nordic Nutrition Recommendations, NNR 2012 [35]

‡ including products made of the related food group

¨All; n=201, men; n=83, women; n=118 after exclusion of those who did not eat fish

^ All; n=180, men; n=76, women; n=104 after exclusion of those who did not eat poultry

§ Eat 6 a day - equivalent to about 600 g vegetables and fruit. At least half should be vegetables

‖ Vegetables are classified from type of food groups (e.g. all types of cabbage, rooted vegetables and onions are classified as coarse vegetables and all vegetables with a high water content like tomatoes and salad are classified as fine vegetables).

¶ All; n=194, men; n=81, women; n=113 after exclusion of those who did not drink alcohol.

4.3.1 Associations between DQI and cardiometabolic risk factors

Table 7 summarises the associations between DQI score and cardiometabolic risk factors in the study population. Only results from the multivariate model 1b (adjusted for sex, age, education, and physical activity during leisure time) are presented. For the results of the simple linear regression model (model 1a, only adjusted for sex and age) and the multivariate model (model 2, additionally adjusted for BMI), see paper I.

Overall, an inverse association was found between DQI and the lipid risk factors: LDL/HDL ratio and TAG (−0.089 per unit, 95% CI −0.177 to −0.002, p = 0.031 and −5% per unit, 95% CI −9% to 0%, p = 0.029, respectively); DQI was positively associated with HDL cholesterol (0.047 mmol/L per unit, 95% CI 0.007 to 0.088 mmol/L, p = 0.021).

For men, the DQI was inversely associated with BMI (−3% per unit, 95% CI −5% to −1%, p = 0.012), trunk fat (−1% per unit, 95% CI −2% to −1%, p = 0.001), hsCRP (−30% per unit, 95% CI −41% to −16%, p = 0.002), HbA1c (−0.09% per unit, 95% CI −0.14% to −0.04%, p < 0.001), insulin (−13% per unit, 95% CI −19% to −7%, p < 0.001), and HOMA-IR (−14% per unit, 95% CI −21% to −7%, p < 0.001). In women, DQI was positively associated with systolic blood pressure (2.6 mmHg per unit, 95% CI 0.6 to 4.6 mmHg, p = 0.021).

Table 7 Linear regression of the associations between adherence to the Danish official dietary guidelines assessed by a DQI and cardiometabolic risk factors in an adult Danish population with minimum one self-reported risk factor for IHD (β-coefficients per unit DQI and 95% confidence intervals (CI), n=219).

	Model 1b† β (95% CI)	
Metabolic markers		
BMI (kg/m^2) (%)	0	(-1, 2)
Men	-3**	(-5, -1)
Women	0	(-1, 2)
Waist/hip-ratio (%)	-1	(-1, 0)
Trunk fat (%)	0	(-1, 1)
Men	-1***	(-2, -1)
Women	0.02	(-0.69, 0.73)
Systolic BP (mm Hg)¨	1.15	(-0.36, 2.67)
Men	-0.68	(-3.04, 1.68)
Women	2.6*	(0.63, 4.58)
Diastolic BP (mmHg)¨	0.92	(-0.3, 2.13)
Men	-1.33	(-2.78, 0.12)
Women	0.92	(-0.3, 2.13)
hsCRP (mg/l) (%)^	-16**	(-25, -6)
Men	-30***	(-41, -16)
Women	-5	(-18, 9)
Lipid biomarkers §		
Total cholesterol (mmol/l)	-0.009	(-0.104, 0.087)
LDL-c (mmol/l)	-0.027	(-0.110, 0.055)
HDL-c (mmol/l)	0.047*	(0.007, 0.088)
<50 years		
>50 years		
LDL/HDL-ratio	-0.089*	(-0.177, -0.002)
VLDL-c (mmol/l) (%)	-5	(-9, 0)
TAG (mmol/l) (%)	-5*	(-9, 0)
Glycaemic biomarkers #		
Glucose (mmol/l) (%)	0	(-1, 1)
HbA_{1c} (%)	0.02	(-0.02, 0.06)
Men	-0.09***	(-0.14, -0.04)
Women	0.02	(-0.02, 0.06)
Insulin (pmol/L) (%)	-2	(-7, 5)
Men (%)	-13***	(-19, -7)
Women (%)	-7	(-7, 5)
HOMA-IR (%)	-1	(-7, 6)
Men (%)	-14***	(-21, -7)
Women (%)	-1	(-7, 6)

DQI, diet quality index; IHD, Ischaemic heart disease; BMI, Body Mass Index; BP, blood pressure; hsCRP, high-sensitivity C-reactive protein; LDL-c, low density lipoprotein-cholesterol; HDL-c, high density lipoprotein-cholesterol; VLDL-c, very low density lipoprotein-cholesterol; TAG, triglycerides; HbA_{1c} , haemoglobin A_{1c}; HOMA-IR, homeostatic model of insulin resistance

Levels of significance are marked as: * p<0.05, ** p=<0.01, *** p=0.001

† Multiple linear regression analysis adjusted for sex, age, education, physical activity at leisure time

‡ Multiple linear regression analysis adjusted for sex, age, education, physical activity at leisure time and BMI

¨ n = 216, after exclusion of those using BP-lowing medication

^ n = 204 due to lack in biochemical analyses of hsCRP

§ n=214 at baseline, n=196 at 6 month and n=193 at 12 month after exclusion of those using cholesterol lowering medication

n=218 at baseline, 201 at 6 month and n=195 at 12 month as it was not possible to draw enough blood for the glycaemic biomarkers analysis

4.4 Results of paper II

4.4.1 Dietary differences from baseline to 6 months

Table 8 presents the between-group differences for changes in dietary intake endpoints from baseline to 6 months and from baseline to 12 months. Only results from the main model, the multiple liner regression model (model 2, adjusted for baseline intake of the outcome variables, sex, age group, and BMI) are presented. For results of the simple model, see paper II in **Appendix A**.

4.4.1.1 Targeted substitution dietary guidelines compared with the habitual diet

Compared with the habitual diet group, the group following the targeted substitution dietary guidelines increased their intakes of whole grains (17 g/10 MJ/d, 95% CI 6 to 28 g/10 MJ/d, p = 0.002), dietary fibre (men: 0.31 g/MJ/d , 95% CI 0.04 to 0.58 g/MJ/d, p = 0.045 and women: 0.23 g/MJ/d, 95% CI 0.002 to 0.45 g/MJ/d, p = 0.024), fine vegetables (41 g/10 MJ/d, 95% CI 5 to 77 g/10 MJ/d, p = 0.024), and decreased their percentage of energy intake from SFA (−1.51 E%, 95% CI −2.31 to −0.70 E%, p < 0.001). In addition, women in the group increased their overall intake of vegetables (69.98 g/10 MJ/d, 95% CI 19.37 to 120.58 g/10 MJ/d, p = 0.007), and normal weight participants in the group increased their E% intake from PUFAs (1.55 E%, 95% CI 0.73 to 2.36 E%, p < 0.001).

4.4.1.2 Danish Official dietary guidelines compared with the habitual diet

Compared with the habitual diet, the group following the Danish official dietary guidelines decreased their E% intake from SFA (−0.89 E%, 95% CI −1.69 to −0.09 E%, p = 0.029). Furthermore, the women in the group increased their overall intake of vegetables (60.31 g/10 MJ/d, 95% CI 10.16 to 110.45 g/10 MJ/d, p = 0.019). Men in the group decreased their intake of coarse vegetables (−40 g/10 MJ/d, 95% CI −78 to −2 g/10 MJ/d, p = 0.040).

4.4.2 Dietary differences from baseline to 12 months

4.4.2.1 Targeted substitution dietary guidelines compared with the habitual diet

Compared with the habitual diet, the group following the targeted substitution dietary guidelines continued to have an increased intake of whole grains from baseline to 12 months (16 g/10 MJ/d,

95% CI 6 to 27 g/10 MJ/d, p = 0.002) and increased intake of fish (23 g/10 MJ/d, 95% CI 3 to 43 g/10 MJ/d, p = 0.022). Obese study participants in the group increased their vegetable intake in general (166 g/10 MJ/d, 95% CI 45 to 287 g/10 MJ/d, p = 0.007). Participants in the group increased their intake of sugar and candy (9 g/10 MJ/d, 95% CI 0.2 to 17 g/10 MJ/d, p = 0.046) and those aged ≥ 50 years increased their E% intake from carbohydrates (2.67 E%, 95% CI 0.15 to 5.19 E%, p = 0.038).

4.4.2.2 Danish official dietary guidelines compared with the habitual diet

Compared with the habitual diet, from baseline to 12 months, the group following the Danish official dietary guidelines had increased intakes of whole grains (13 g/10 MJ/d, 95% CI 3 to 23 g/10 MJ/d, p = 0.012) and fish (24 g/10 MJ/d, 95% CI 5 to 44 g/10 MJ/d, p = 0.016), and they continued to have a decreased E% intake from SFA (−0.84 E%, 95% CI −1.69 to −0.001 E%, p = 0.050). Participants aged ≥ 50 years in the group increased their E% intake from carbohydrates (2.95 E%, 95% CI 0.45 to 5.46 E%, p = 0.021).

4.4.3 Differences in DQI score from baseline to 6 and 12 months

Table 9 presents the between-group differences in changes of DQI score from baseline to 6 months and from baseline to 12 months (not included in paper II).

Compared with the habitual diet group, both the group receiving the targeted substitution dietary guidelines and the group receiving the Danish official dietary guidelines increased their DQI scores from baseline to 6 months (0.6 units, 95% CI 0.2 to 1.0 units and 0.4 units, 95% CI 0.02 to 0.9 units, respectively) and from baseline to 12 months (0.7 units, 95% CI 0.3 to 1.2 units and 0.9 units, 95% CI 0.4 to 1.3 units, respectively).

4.4.4 Within group differences in dietary intake endpoints and IHD risk factors

In **Tables 2** and **3** of **Appendix B**, mean changes (95% CI) in dietary data and IHD risk factors in the randomised groups, from baseline to 6 months and from baseline to 12 months are presented (not included in paper II).

4.4.5 Association between whole grain intake and plasma alkylresorcinols at baseline

From the cross-classification between reported whole grain intake and alkylresorcinol concentrations, 36% of study participants were classified in the same quartile, 70% were classified in the same or an adjacent quartile, 22% were two quartiles apart, and 9% were misclassified in the

opposite quartile (data not shown). A statistically significant association between whole grain intake and plasma alkylresorcinols was observed at baseline ($p < 0.0001$).

4.5 Results of paper III

4.5.1 Differences in IHD risk factors

In **Table 10** the between-group differences in IHD risk factors from baseline to 6 months and from baseline to 12 months are presented. Only results from the main model, the multiple liner regression model (model 2, adjusted for baseline intake of the outcome variables, sex, age group, and BMI) are presented. For results from the simple model, see paper III in **Appendix A**.

Overall, no differences were found in cardiometabolic risk factors for either of the two dietary guideline groups when compared with the habitual diet. A significant decrease was found in waist circumference (−4.41 cm, 95% CI −7.93 to −0.88 cm, $p = 0.015$) from baseline to 12 months among obese study participants following the Danish official dietary guidelines, compared with the habitual diet group.

Table 8 Between-group differences in changes in energy adjusted diet composition (g/10 MJ/d), total energy (MJ/d), energy contribution (E%) of macronutrients, dietary fibre (g/MJ) from baseline to 6 months and from baseline to 12 months (means and 95% confidence intervals (CI), n=219 at baseline, n= 199 at 6 month and n= 186 at 12 months).

	Model 2† From baseline to 6 months				Model 2† From baseline to 12 months			
	SUB DG vs. habitual Mean between group difference, 95%CI		OFF DG vs. habitual Mean between group difference, 95%CI		SUB DG vs. habitual Mean between group difference, 95%CI		OFF DG vs. habitual Mean between group difference, 95%CI	
Diet composition (g/10 MJ/d):								
Bread and cereals	6	(-14, 26)	-2	(-22, 18)	10	(-10, 31)	7	(-14, 27)
Whole grains	18**	(7, 28)	7	(-4, 17)	16**	(6, 27)	13*	(3, 23)
Meat and meat products	-16	(-39, 6)	-15	(-38, 7)	-22	(-47, 2)	-24	(-48, 0.3)
Poultry and poultry products	-4	(-36, 27)	-10	(-39, 20)	2	(-17, 20)	15	(-3, 33)
Fish and fish products	12	(-7, 30)	15	(-4, 33)	23*	(3, 43)	24*	(5, 44)
Fruit and fruit products	26	(-12, 64)	23	(-15, 62)	-4	(-42, 34)	-1	(-39, 36)
Vegetables and vegetables products	45*	(5, 84)	16	(-23, 55)	-20	(-69, 30)	-11	(-61, 38)
Women	70**	(19, 121)	60*	(10, 110)				
Men	8	(-52, 69)	-48	(-109, 12)				
Normal weight					-92	(-183, 0.1)	-71	(-156, 15)
Overweight					-36	(-101, 29)	6	(-60, 72)
Obese					166**	(45, 287)	61	(-62, 185)
Vegetables, coarse‡	9	(-15, 334)	-3	(-27, 22)	-14	(-45, 17)	-8	(-38, 23)
Women	18	(-14, 50)	23	(-9, 54)				
Men	-4	(-42, 34)	-40*	(-78, -2)				
Vegetables, fine‡	41*	(5, 77)	34	(-2, 70)	-8	(-39, 24)	-9	(-41, 22)
Potatoes and potatoes products	-9	(-30, 11)	5	(-15, 25)	-7	(-33, 20)	14	(-11, 39)
Women					14	(-20, 48)	4	(-28, 36)
Men					-37	(-77, 3)	29	(-8, 66)
Milk and milk products	-6	(-65, 53)	38	(-21, 96)	19	(-33, 71)	-4	(-54, 47)
Cheese and cheese products	-18	(-37, 1)	-18	(-36, 1)	-23	(-48, 2)	-4	(-29, 21)
Edible fats	-4	(-8, 1)	-3	(-7, 2)	0.1	(-4, 5)	-2	(-6, 3)
Sugar and candy	5	(-3, 13)	3	(-5, 11)	9*	(0.2, 17)	3	(-5, 12)
Total energy, energy contribution of macronutrient, dietary fibre								

Energy, MJ	-0.84	(-3.73, 2.05)	1.53	(-1.28, 4.34)	0.13	(-0.45, 0.71)	0.31	(-0.27,.89)
Energy from protein, %	-0.50	(-1.34, 0.34)	-0.17	(-1.01, 0.66)	-0.44	(-1.46 , 0.57)	0.42	(-0.59, 1.43)
Energy from carbohydrate, %	1.77	(-0.23, 3.78)	1.83	(-0.16, 3.81)	1.03	(-0.95, 3.02)	0.90	(-1.07, 2.87)
<50 years					-1.29	(-4.40, 1.83)	-2.18	(-5.28, 0.92)
≥50 years					2.67*	(0.15, 5.19)	2.95*	(0.45, 5.45)
Energy from added sugar, %	0.02	(-1.08, 1.12)	0.09	(-1.01, 1.19)	0.86	(-0.24, 1.96)	0.07	(-1.02, 1.17)
Energy from total fat, %	-1.35	(-3.05, 0.36)	-1.03	(-2.72, 0.66)	0.05	(-1.60, 1.69)	-0.63	(-2.26, 1.00)
Energy from SFA, %	-1.51***	(-2.3, -0.70)	-0.89*	(-1.69, -0.09)	-0.73	(-1.59, 0.12)	-0.84*	(-1.69, -0.001)
Energy from MUFA, %	-0.22	(-1.13, 0.70)	-0.19	(-1.10, 0.72)	0.44	(-0.51, 1.38)	0.06	(-0.87, 1.00)
Energy from PUFA, %	0.47*	(0.04, 0.91)	0.03	(-0.40, 0.46)	0.19	(-0.24, 0.61)	0.24	(-0.18, 0.66)
Normal weight	1.55***	(0.73, 2.36)	0.05	(-0.71, 0.81)				
Overweight	-0.06	(-0.64, 0.51)	-0.03	(-0.61, 0.55)				
Obese	0.60	(-0.42, 1.63)	0.11	(-0.95, 1.18)				
Energy from alcohol, %	0.50	(-0.67, 1.67)	-0.48	(-1.67, 0.70)	-1.15	(-2.37, 0.07)	-1.11	(-2.33, 0.11)
Dietary fibre, g/MJ/d	0.26**	(0.09, 0.44)	0.08	(-0.09, 0.25)	0.08	(-0.10, 0.26)	0.10	(-0.08, 0.28)
Women	0.23*	(0.01, 0.45)	0.22	(-0.01, 0.44)				
Men	0.31*	(0.04, 0.58)	-0.12	(-0.39, 0.15)				

Abbreviations: SUB DG, targeted substitution dietary guidelines; habitual, habitual diet; OFF DG, Danish official dietary guidelines; g, gram; MJ, mega joule; SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

Levels of significance are marked as: * p<0.05, ** p=<0.01, *** p=0.001

†multiple liner regression model adjusted for baseline intake of the outcome variable, sex, age group (<50 and ≥50), BMI group (18.5-25 = Normal weight, >25-30 = Overweight, >30 = Obese) and interactions between intervention group and sex, intervention group and age group, and intervention group and BMI group

‡ Vegetables are classified from type of food groups (e.g. all types of cabbage, rooted vegetables and onions are classified as coarse vegetables and all vegetables with a high water content like tomatoes and salad are classified as fine vegetables).

Table 9 Between-group differences in changes in DQI score from baseline to 6 months and from baseline to 12 months (means and 95% confidence intervals (CI), n=219 at baseline, n= 199 at 6 month and n= 186 at 12 months).

	Model 2† From baseline to 6 months				Model 2† From baseline to 12 months			
	SUB DG vs. habitual Mean between group difference, 95%CI		OFF DG vs. habitual Mean between group difference, 95%CI		SUB DG vs. habitual Mean between group difference, 95%CI		OFF DG vs. habitual Mean between group difference, 95%CI	
DQI score	0.6**	(0.2, 1.0)	0.4*	(0.02, 0.9)	0.7**	(0.3, 1.2)	0.9***	(0.4, 1,3)

Abbreviations: DQI, Diet quality index; SUB DG, targeted substitution dietary guidelines; habitual, habitual diet; OFF DG, Danish official dietary guidelines

Table 10 Between-group differences in cardio-metabolic risk factors from baseline to 6 month and from baseline to 12 months (means and 95% confidence intervals (CI), n=219 at baseline, n= 203 at 6 month and n= 196 at 12 months).

	Model 2† From baseline to 6 months				Model 2† From baseline to 12 months			
	SUB DG vs. habitual Mean between group difference, 95%CI		OFF DG vs. habitual Mean between group difference, 95%CI		SUB DG vs. habitual Mean between group difference, 95%CI		OFF DG vs. habitual Mean between group difference, 95%CI	
Weight (kg)	-0.40	(-1.40, 0.60)	-0.67	(-1.67, 0.32)	-0.19	(-1.40, 1.03)	-0.47	(-1.68, 0.73)
BMI (kg/m^2)	-0.13	(-0.47, 0.20)	-0.24	(-0.58, 0.09)	-0.06	(-0.47, 0.35)	-0.17	(-0.58, 0.23)
Waist circumference (cm)	-0.29	(-1.62, 1.05)	0.11	(-1.22, 1.45)	-0.92	(-2.33, 0.48)	-0.08	(-1.49, 1.32)
Normal weight	-0.27	(-2.75, 2.20)	-1.07	(-3.44, 1.30)	-0.94	(-3.55,1.67)	-0.43	(-2.88, 2.02)
Overweight	-1.14	(-2.93, 0.64)	1.13	(-0.67, 2.93)	-1.31	(-3.15, 0.53)	1.27	(-0.58,3.13)
Obese	2.45	(-0.67, 5.57)	-0.98	(-4.19, 2.24)	0.28	(-3.06, 3.63)	-4.41***	(-7.93,-0.88)
Hip circumference (cm)	-0.61	(-1.64, 0.41)	-0.09	(-1.11, 0.93)	-0.07	(-1.24, 1.11)	-0.27	(-1.43, 0.90)
Normal weight					0.06	(-2.15, 2.27)	-1.20	(-3.27, 0.86)
Overweight					-0.8	(-2.34, 0.76)	0.46	(-1.10, 2.03)
Obese					2.27	(-0.56, 5.11)	-1.05	(-3.99, 1.90)
Waist/hip-ratio	0.003	(-0.009, 0.02)	-0.00009	(-0.01, 0.01)	-0.007	(-0.02, 0.005)	0.0008	(-0.01, 0.01)
Body fat (%)	0.08	(-0.54, 0.70)	-0.10	(-0.72, 0.5)	0.59	(-0.20, 1.38)	-0.04	(-0.82, 0.74)
Trunk fat (%)	0.12	(-0.62, 0.87)	-0.12	(-0.86, 0.62)	0.75	(-0.16, 1.67)	-0.03	(-0.93, 0.88)
Systolic BP (mm Hg)‡	1.25	(-1.95, 4.45)	-1.28	(-4.46, 1.89)	-0.42	(-3.69 2.86)	-2.99	(-6.26, 0.28)
Diastolic BP (mm Hg)‡	1.37	(-0.32, 3.05)	-0.73	(-2.40, 0.93)	0.24	(-1.63, 2.12)	-1.22	(-3.09, 0.65)
hsCRP (mg/L)^	0.18	(-0.23, 0.58)	-0.04	(-0.44, 0.36)	0.07	(-0.4, 0.60)	-0.03	(-0.56, 0.50)

Lipid biomarkers§								
Total cholesterol	-0.12	(-0.29, 0.06)	-0.08	(-0.26 0.10)	0.07	(-0.17, 0.32)	-0.14	(-0.38, 0.10)
LDL-HDL ratio	0.007	(-0.14, 0.16)	0.04	(-0.11, 0.19)	0.06	(-0.13, 0.26)	-0.06	(-0.25, 0.14)
LDL cholesterol (mmol/L)	-0.08	(-0.23, 0.08)	-0.03	(-0.19, 0.12)	0.12	(-0.10, 0.33)	-0.10	(-0.31, 0.11)
HDL cholesterol (mmol/L)	-0.001	(-0.06, 0.06)	-0.01	(-0.07, 0.05)	0.03	(-0.04, 0.10)	-0.01	(-0.08, 0.07)
VLDL cholesterol (mmol/L)	-0.04	(-0.12, 0.04)	-0.02	(-0.10, 0.07)	-0.06	(-0.16, 0.04)	-0.01	(-0.11, 0.08)
TAG (mmol/L)	-0.09	(-0.28, 0.10)	-0.03	(-0.22, 0.15)	-0.15	(-0.37, 0.07)	-0.04	(-0.25, 0.18)
Glycaemic biomarkers§								
Glucose (mmol/L)	0.06	(-0.05, 0.18)	0.03	(-0.08, 0.15)	-0.01	(-0.13, 0.10)	0.04	(-0.07, 0.16)
eAG (mmol/L)	-0.008	(-0.13, 0.12)	0.014	(-0.11, 0.14)	0.001	(-0.11, 0.12)	0.09	(-0.02, 0.21)
HbA1c (%)	-0.001	(-0.08, 0.07)	0.01	(-0.07, 0.08)	0.003	(-0.07, 0.08)	0.06	(-0.01,0.13)
HOMA-IR	-0.06	(-0.44, 0.32)	-0.04	(-0.42, 0.34)	-0.08	(-0.48, 0.32)	0.11	(-0.29, 0.51)
Insulin (pmol/L)	-2.56	(-12.37, 7.25)	-2.29	(-12.00, 7.42)	-2.09	(-11.66, 7.48)	3.58	(-5.95, 13.10)
C-peptid	-2.49	(-41.32, 6.34)	1.04	-37.49, 39.58)	1.01	(-38.52, 40.54)	39.00	(-0.45, 78.46)

Abbreviations: SUB DG, targeted substitution dietary guidelines; habitual, habitual diet; OFF DG, Danish official dietary guidelines; BMI, Body Mass Index; BP, blood pressure; hsCRP, high-sensitivity C-reactive protein; LDL, low density lipoprotein-cholesterol; HDL, high density lipoprotein-cholesterol; VLDL, very low density lipoprotein-cholesterol; TAG, triglycerides; HbA_{1c}, haemoglobin A_{1c}; HOMA-IR, homeostatic model of insulin resistance

† multiple liner regression model adjusted for baseline intake of the outcome variable, sex, age group (<50 and ≥50), BMI group (18.5-25 = Normal weight, >25-30 = Overweight, >30 = Obese) and interactions between intervention group and sex, intervention group and age group, and intervention group and BMI group

‡ n=216 at baseline, n= 201 at 6 month and n= 190 at 12 month after exclusion of those using BP-lowering medication

¨ n= 204 at baseline, n=196 at 6 month and n= 185 at 12 month due to lack in biochemical analysis of hsCRP

^ n=214 at baseline, n=196 at 6 month and n=193 at 12 month after exclusion of those using cholesterol lowering medication

§ n=218 at baseline, 201 at 6 month and n=195 at 12 month as it was not possible to draw enough blood for the glycaemic biomarkers analysis

4.6 Under-and over-reporting and medication users (papers I to III)

4.6.1 Under- and over-reporters

At baseline, at 6 months, and at 12 months, 21%, 35%, and 31% of the participants, respectively, were classified as under-reporters. In addition, 1% were classified as over-reporters at baseline, 6 months, and 12 months. Of the under-reporters, 86%–90% were overweight or obese, and 41%–44% were men; all the over-reporters were men.

In paper I, the associations between DQI and most of the included variables were the same whether under- and over-reporters were or were not included in the analysis. In paper II, excluding under- and over-reporters for the sensitivity analysis did not change the findings for the two sets of dietary guidelines, compared with the habitual diet.

4.6.2 Medication users

Participants who were taking medications to lower cholesterol (n = 5 at baseline, n = 7 at 6 months, and n = 3 at 12 months) and/or blood pressure (n = 3 at baseline, n = 2 at 6 months, and n = 6 at 12 months) were excluded from the statistical analysis of lipid biomarkers and blood pressure, respectively.

5 Discussion

5.1 Main results

Firstly, in the cross-sectional study of paper I, a closer adherence to the current Danish official dietary guidelines, assessed by a DQI, was found to be associated with a more beneficial cardiometabolic risk profile in an adult Danish population with a minimum of one self-reported risk factor for IHD. The overall results showed an inverse association between DQI score and lipid risk factors (LDL/HDL ratio and TAG) and a positive association with HDL cholesterol. For male participants, an inverse association between DQI and BMI, trunk fat, hsCRP, and glycaemic risk factors was found. Furthermore, for women, we found a positive association between DQI and systolic blood pressure.

Secondly, the main results of the DIPI RCT showed that, compared with the habitual diet, during the intervention period from baseline to 6 months, the targeted substitution dietary guidelines were more effective than the Danish official dietary guidelines in changing the number of dietary components, resulting in a dietary composition of the overall diet that was more cardioprotective. However, when including follow-up, from baseline to 12 months, the effectiveness in changing the dietary composition in this manner was similar for both the targeted substitution dietary guidelines and the Danish official dietary guidelines, compared with the habitual diet (paper II). This was supported by the observed positive changes in DQI score, indicating an increased adherence to both sets of dietary guidelines throughout the intervention and follow-up periods, compared with the habitual diet.

Even though both sets of dietary guidelines were effective in changing dietary composition towards a more cardio-protective dietary pattern, neither applying the targeted substitution dietary guidelines nor the Danish official dietary guidelines showed any overall effects on the included intermediate risk factors for IHD, from baseline to 6 or 12 months (paper III).

5.2 Paper 1: Comparison with other observational studies

The findings of the cross-sectional study of paper I are in line with those of the cross-sectional study by Toft et al. including 6542 healthy adult Danish men and women, comparable with the study population of the DIPI RCT. In the study Toft et al. found that a higher DQS, indicating closer adherence to the 2005 Danish official dietary guidelines, was associated with a more beneficial CVD risk factor profile [(90)].

The observed positive associations between a higher dietary score and better cardiometabolic risk profile found in both paper I and in the study by Toft et al. could potentially be used for prevention of cardiovascular-related diseases. This is supported by findings of the recently published prospective cohort study of Hansen et al. including 55,021 adult Danish men and women [95]. In that study, greater adherence to the 2013 Danish official dietary guidelines, assessed by another updated DQI called the Danish Guidelines Index Score, was associated with a 13% and 24% lower risk of MI in men and women, respectively.

The sex-specific inverse associations between DQI and BMI, trunk fat, hsCRP, and glycaemic risk factors for male participants found in paper I are also in line with the results of two cohort studies, by Drewnowski et al. [123] and Frazier-Wood et al., both investigating the association between diet quality, as indexed by the HEI, originally developed to assess adherence to the Dietary Guidelines for Americans, and cardiovascular risk factors [124].

In the cross-sectional study by Drewnowski et al. including 5,081 middle-aged French men and women with low CVD risk, an inverse association between HEI and BMI and blood pressure was found in men only [123]. Also in the cross-sectional study by Frazier-Wood et al.[124] including 9,797 adult American men and women with at least one CVD risk factor, comparable with the study population of the present research, the HEI score was inversely associated with insulin, HOMA-IR, HDL cholesterol, TAG, and CRP in men only [125]. Yet, in contrast to our results, when Frazier-Wood et al. adjusted for BMI, the results were no longer significant. In paper I, when further adjustments for BMI were made, the associations between DQI score and glycaemic biomarkers remained significant; however, the associations between DQI score and LDL/HDL ratio, TAG, and trunk fat were attenuated and no longer statistically significant. These results and those of Frazier-Wood et al. suggest that BMI might be an important factor in cardiovascular disease prevention [125].

The sex difference of inverse associations between DQI and BMI, trunk fat, hsCRP, and glycaemic risk factors for male participants found in the results of paper I, could be explained by the higher number of overweight men than women. In addition, we only found an inverse significant association between DQI and BMI in men. Also, the link between BMI, trunk fat, and hsCRP is supported by the recognised inverse relationship between BMI and CRP. Moreover, it is known that CRP is further elevated with increased adiposity [126].

5.2.1 Methodological considerations: Paper I, the cross-sectional study

5.2.1.1 The DQI

In contrast to the more traditional approach of focusing on single foods and nutrients, use of the DQI to assess adherence to the Danish official dietary guidelines and the associations with IHD risk factors allowed us to combine information on multiple dietary data into this single indicator of adherence and to catch potential additive or synergistic effects of the different components of the diet.

The predefined DQI is based on five food and nutrient guidelines of the 10 Danish official dietary guidelines. Originally, the DQI was developed for the purpose of evaluating the overall diet quality based on the 2005 Danish official dietary guidelines and not for evaluation of the potential health effects of the included foods and nutrients [91]. Therefore, the items included in the DQI are not weighted according to their expected health effects; it is therefore not plausible to expect an association with all the outcomes included in the present study.

In addition, the DQI does not include intakes of meat and salt, which have both been associated with low cardiometabolic health [52, 54, 127–130]. Especially, an excessive intake of salt/sodium have been associated with an elevated blood pressure in some people [128–130], and intake of salt could be related to the surprising and unexpected finding of a positive association between the DQI and systolic blood pressure in women, which is not in line with previous studies of dietary patterns and blood pressure. In a meta-analysis of 17 RCTs investigating the effect of dietary patterns on blood pressure in adults, it was found that healthy dietary patterns such as those of the Nordic, Mediterranean, and DASH diets decreased systolic and diastolic blood pressure [131]. The primary reason that salt was not included in the DQI is that estimation of sodium chloride with the dietary record method that was used to collect dietary intake information would involve excessive uncertainty.

However, the index used in the present study includes whole grains, fish, fruits and vegetables, energy from saturated fat, and energy from added sugar, all of which have been associated with either a reduced risk of IHD or an increased risk of IHD [5, 43, 64, 69, 132]. Therefore, it was expected that a higher DQI score would be associated with a better cardiometabolic risk profile.

5.2.1.2 Internal and external validity

The results of paper I were based on baseline data from the DIPI RCT, of which the internal and external validity is discussed in details in section 5.3.1.

A strength of this cross-sectional study was the availability of detailed dietary intake data, collected using a web-based 7-day dietary record, limiting measurement errors. In addition, sub-analysis excluding under- and over-reporters was also conducted, which made it possible to consider the potential information-bias of under- and over-reporting in the self-reported dietary data. Furthermore, different liner regression models were applied in order to consider potential confounders; unmeasured confounding cannot be ruled out, however. In addition, interaction effects were examined to determine whether the associations between DQI and the outcome variables were different between sexes and age groups.

A limitation of this study was the observational design of the cross-sectional study, which does not allow for conclusions to be made about cause and effect relationships. Furthermore, the power of the study, which was originally calculated for the DIPI RCT, must be considered quite low for a cross-sectional study, with only 219 included study participants. Considering these limitations, and especially the fact that the study was underpowered, both the internal and external validity of the study must be considered quite low, and generalisation based on the results should be made with caution.

5.3 Paper II and III: Comparison with other dietary RCT

The results of the DIPI RCT are in line with results of the previously described RCT by Jenkins et al. [104]. Comparable with the findings of increased whole grain intake in the DIPI RCT, Jenkins et al. also observed an increase in whole grain intake in their intervention group receiving only dietary advice. However, in contrast to the DIPI RCT, Jenkins et al. only observed increased intake of other recommended foods in the intervention groups that were provided with free foods; they did not note any other changes in dietary intake endpoints in the intervention group only receiving dietary advice. Nevertheless, in line with results of the present study, Jenkin et al. did not find any short- or long-term difference in CVD risk factors among any of the three intervention groups, in comparison with the control group.

Two other previously presented dietary RCTs, both conducted in a real-life setting, comparable to the DIPI RCT are the CRESSIDA study by Reidlinger et al. [105] and the SYSDIET study by

Uusitupa et al. [106]. Like the DIPI RCT, these studies found a beneficial dietary effect of both the UK dietary guidelines, which are broadly similar to the Danish official dietary guidelines, and the healthy Nordic diet. However, in contrast to the DIPI RCT and the RCT by Jenkins et al. [104] both the study by Reidlinger et al. [105] and that by Uusitupa et al. [106] found beneficial effects of providing advice on selected CVD risk factors, potentially leading to prevention of CVD.

In contrast to the DIPI RCT, in a sub-study of the aforementioned long-term dietary RCT, the PREDIMED study, Estruch et al. found short-term beneficial effects on CVD risk factors in both groups following the two Mediterranean diets that were provided with either olive oil or nuts, in comparison with a low-fat diet [120]. The sub-study included 772 participants and measured dietary compliance and CVD risk factors after 3 months. Apart from an increase in E% intake from the provided foods, Estruch et al. observed an increase in E% intakes from total fat, MUFAs, and PUFAs, and a decrease in E% intake from carbohydrates in the Mediterranean diet group provided with nuts, compared with the low-fat group. Estruch et al. did not report other dietary changes in either of the two Mediterranean diet groups, in comparison with the low-fat diet. However, long-term beneficial dietary changes were found in both Mediterranean diet groups provided with olive oil or nuts, and a concomitant 30% and 28% lower risk of major cardiovascular events, respectively, compared with the low-fat diet group [107].

In the studies by Reidlinger et al. [105], Uusitupa et al. [106], and Estruch et al. [107, 120], the primary dietary changes were found in fat subtypes, all of which reflect the fat content of the provided food items. When compared with a control group, Reidlinger et al. [105] found a 7.2 E% decrease from SFA and a 1.9 E% increase from PUFAs in the group following the UK dietary guidelines. In addition, Uusitupa et al. [106] found a decrease of 4.3 E% from SFA and an increase of 2.1 E% from PUFAs in the group following the healthy Nordic diet, when compared to the control group. In the PREDIMED study, Estruch et al. found short- and long-term increases of 3.0 E% and 2.0 E% from PUFAs, respectively, in the group following a Mediterranean diet plus nuts, and a long-term increase of 0.2 E% from SFA and 0.6 E% from PUFAs for those on the Mediterranean diet plus olive oil, when compared with the group following the low-fat diet [107, 120].

In the DIPI RCT, when compared with the control group, the short-term changes in fat subtypes in both the group following the targeted substitution dietary guidelines and in the group following the Danish official dietary guidelines were a decrease in energy percentage from SFA of 1.5 E% and 0.9 E%, respectively. In addition, normal-weight participants in the targeted substitution dietary

guideline group increased their energy percentage from PUFAs with 1.6 E%. The long-term changes in fat subtypes were that the group assigned to follow the Danish official dietary guidelines decreased their energy percentage from SFA with 0.8 E%, when compared with the control group.

The differences in dietary intake (including fat subtypes) between the DIPI RCT and the three above-mentioned studies by Reidlinger et al. [105], Uusitupa et al. [106], and Estruch et al. [107, 120] (all of which found an effect of the investigated dietary guidelines or healthy diets on CVD risk factors and major cardiovascular events) might be due to the fact that, contrary to the present study, the participants in the other studies were all provided with key foods according to UK dietary guidelines, the healthy Nordic diet, or the Mediterranean diet [105–107, 120]. This provision of key foods might also be the most important difference between these studies and the DIPI RCT.

The inconsistency between results of the DIPI RCT and the studies by Reidlinger et al. [105], Uusitupa et al. [106], and Estruch et al. [107] might also be due to other differences in the intensity of the interventions, apart from the provision of key foods. The intervention/communication format used in the DIPI RCT (leaflets, a website including information on dietary guidelines and recipes, and biweekly e-mails with new recipes) was chosen to reflect how information about dietary guidelines is normally transmitted in real life. Contrary to this, in the study by Jenkins et al., the group that only received dietary advice was provided with leaflets but also had 20–30 minutes of telephone interviews weekly in the first months and monthly in the final 5 months of the intervention [104]. Likewise, adherence to both the UK dietary guidelines investigated by Reidlinger et al. [105] and the Mediterranean diet investigated by Estruch et al. [107] was achieved through dietary advice provided in face-to-face meetings with a dietitian. Both telephone interviews and face-to-face meetings might be assumed to be more motivating informational methods for study participants than the less-interactive media used in the present study [105, 107]. One can speculate that if similar intervention tools had been utilized in the DIPI RCT, this might have resulted in a higher adherence to the dietary guidelines and a concomitant effect on the risk factors for IHD.

That said, the effect of the intensity of the intervention was exactly what Jenkin et al. was aiming to test in the previously mentioned study [104]. However, despite including three intervention groups with different intensity (receiving either advice, food provision or advice plus food provision) no measured effect on CVD risk factors was found in either of the groups, when compared to a control group. This indicates that the intensity of the intervention may not impact the results, however, more research is needed in order to draw any conclusions.

The baseline health status of study participants might also be of significance when measuring risk factors for IHD. In the present study, we recruited participants with a minimum of one self-reported risk factor for IHD, but, although most were overweight or obese and had an elevated waist circumference, the study participants were generally healthy non-smokers and were not taking blood pressure-lowering or anti-hyperlipidaemia medication, and the baseline LDL cholesterol levels of the participants were only slightly elevated. This was similar to the study participants in the studies by Jenkins et al. [104] and Reidlinger et. al. [105], where only Reidlinger et al. found an effect of the investigated UK dietary guidelines on CVD risk factors [105]. However, in contrast to the baseline health status of the study participants in the DIPI RCT both the RCTs by Estruch et al. [107, 120] and Uusitupa et al. [106], included men and women with higher cardiovascular risk, and here both studies found a cardioprotective effect of the investigated diets [106, 107, 120]. The differences between the results of the DIPI RCT and these studies may therefore to a certain extent be due to differences in the study participants baseline health status.

The study participants' habitual intake of key foods and nutrients included in both sets of dietary guidelines could also have contributed to the fact that we did not find any changes in IHD risk factors. When compared with the habitual diet, we found the largest dietary changes for intake of whole grains, fish, and SFA in both dietary guideline groups. A higher intake of both whole grains and fish containing high amounts of PUFAs has been associated with reduced risk of IHD [5]. In addition, there is strong evidence that consuming PUFAs in place of SFA reduces IHD [57–59]. However, the participants' median baseline/habitual intake of whole grains and fish was already relatively high, nearly reaching the recommended 75 g/10 MJ/d and 50 g/10 MJ/d, respectively [5]. By comparison, the mean baseline intake of whole grains in the studies by Jenkins et al. [104], Reidlinger et al. [105], and Estruch et al. [107] was only 1.0–1.5 servings/d (1 serving, 30 g) or approximately 30–45 g/d (whole grain intake was not presented in Uusitupa et al.'s study). In a meta-analysis including dose–response analysis of whole grain intake and CVD mortality, a nonlinear effect was found [133]. From the dose–response analysis of that meta-analysis, each 28 g/d of whole grain intake was associated with a 14% lower risk of CVD mortality; however, when comparing the higher range of whole grain intake, the curve appeared steeper at the lower range of whole grain intake [133]. This finding supports the recommendation for a whole grain intake of around 75 g/d.

Conversely, study participants in the DIPI RCT had a median baseline/habitual intake of meat and meat products (prepared weight) that was much higher (139 g/10 MJ/d) than the recommended maximum of 70 g/10 MJ/d [5] and a lower intake of fruit and vegetables (388 g/10 MJ/d) than the recommended 600 g/10 MJ/d [5]. No significant differences in changes for meat or fruit and vegetable intake were observed in the DIPI RCT.

Closer adherence to the dietary guidelines, with an additional significant reduction in meat intake and higher intake of fruit and vegetables, might have led to a change in IHD risk factors. Although too small to have a beneficial effect on IHD risk factors, the dietary changes found in both the targeted substitution dietary guideline and official dietary guideline groups nearly all constituted improvements, compared with the habitual diet. This is also supported by the finding of an increased DQI score in both dietary guidelines groups, compared with the habitual diet group, throughout both the intervention and the follow-up period, indicating a higher adherence to both sets of dietary guidelines.

The results of the above-mentioned dietary RCTs reveal the many challenges that are related to imposing dietary changes, which may influence the disease course of IHD. Therefore, addressing other modifiable risk factors, such as smoking and physical activity, might be a plausible contribution to the prevention of IHD. Several RCTs have been conducted with the purpose of primary prevention of CVD through investigating multiple behavioural risk factors such as smoking cessation, healthy food choices, and increased physical activity [134–137]. However, these multiple risk factor interventions that use counselling and education appear to only have a small effect on CVD risk factors and appear to have no effect on CVD events in the general population [134, 135, 137].

One example of such a large-scale multiple health behaviour change intervention study is the Danish INTER99 study, which investigated the effect of screening and multiple lifestyle counselling on the incidence of IHD and stroke [138]. The study was a 5-year parallel RCT, where study participants were randomly assigned to an intervention group including 6,091 adult men and women and a control group including 3,324 adult men and women. The intervention consisted of counselling based on the participants' lifestyle and degree of IHD risk. Comparable to the results of the DIPI RCT, the INTER99 study also found some favourable changes in dietary endpoints in the intervention group, compared with the control group [139, 140], but no significant difference in IHD or stroke was seen between the intervention and control groups after 10 years [138].

5.3.1 Methodological considerations: Papers II and III, the DIPI RCT

5.3.1.1 Internal validity

5.3.1.1.1 Design

To test the effects of the targeted substitution dietary guidelines we chose a RCT. A key factor in a RCT is the allocation of participants by chance to receive one or several interventions so that the only systematic difference between the groups is the intervention, making the RCT the gold standard with regard to internal validity [141]. In the present RCT no differences in key endpoints were found at baseline, indicating that the randomisation was successfully conducted.

The DIPI RCT was conducted in a real-life setting where participants were free-living and empowered to modify their dietary pattern, which must be considered a strength as it reflects the "real-world" thereby making the application of the results more straight-forward than controlled feeding trials. However, this type of study also increases the chance of the intervention being blurred by confounders.

The design of the DIPI RCT included a standard control group that was not provided with any form of intervention; a primary intervention group that received the targeted substitution dietary guidelines; and a second intervention group that received the Danish official dietary guidelines. This made it possible to investigate the effects of the two intervention groups, when compared to the control group.

Even though the DIPI RCT was designed and conducted to fulfil the criteria for a RCT to the highest possible degree, the study still has some limitations.

First of all, the design of the study made it impossible to blind participants, which may bias the results as this increased the risk of deteriorating motivation within the control group due to dissatisfaction with being assigned to the control group and not receiving any dietary guidelines. At the same time the control group were free to search information elsewhere and had access to the Danish official dietary guidelines via official homepages and other channels. However, as the intervention was conducted only 6 months after the release of the 2013 Danish official dietary guidelines, the assumption was that the study participants were not yet very aware of the updated official dietary guidelines. Supporting this assumption is the fact that only a small decrease in energy intake was observed in the control group from baseline to 6 months, indicating that the

control group did in fact not follow the dietary guidelines on their own accord (**Tables 2** and **3** of **Appendix B**).

Secondly, in the design of the DIPI RCT the power calculation was based on LDL cholesterol, one of the major modifiable risk factors for IHD [142]. However, even though dietary effects of both sets of dietary guidelines compared with the habitual diet was found, only small statistically insignificant differences in changes in LDL cholesterol in both intervention groups, compared with the control group was observed. This "negative/null finding" could be due to insufficient power resulting in type II errors [143, 144]. Freiman et al. reviewed the power calculations of 71 RCTs with "negative/null" results and found that the estimation of the sample size may assume an unrealistically large intervention effect [144]. In the DIPI RCT the intervention effect was set to a difference in LDL cholesterol of 0.25 mml/L. However, retrospectively taking the intensity of the intervention into consideration (further discussed below in section 5.1.1.1.3) this might have been too high.

5.3.1.1.2 The study population: Selection and drop-outs

Due to fact that examinations took place at Gentofte Hospital, recruitment of study participants were limited to the areas of Greater Copenhagen surrounding the hospital, for practical and cost related reasons. In total, 5000 men and women living in the defined area of Greater Copenhagen, who were identified using a unique personal identification number assigned to all Danish citizens in the Civil Registration System [108], were invited by letter to participate in the study. Of the 5000 possible participants invited, only 7% responded to the invitation and were screened, which may induce sample bias.

Inclusion criteria were made to define the target population. The aim was to include an adult population with at least one risk factor for IHD. Due to prohibitive cost, it was not possible to analyse blood samples for lipid biomarkers such as LDL cholesterol, and self-measured risk factors was therefore selected. As it turned out, however, the study participants' self-reported weight and waist circumference were statistically significantly higher than the measured weight and waist circumference at baseline – possibly because the motivation of the responders to be a part of the study was high; however, no difference in BMI was found. This resulted in 17% of women and 7% of men not meeting the inclusion criteria when measured at baseline. Also exclusion criteria were applied to limit the influence of confounding participant characteristics.

A major source of bias in RCTs is related to intervention dropouts. Therefore, in the statistical methods of the DIPI RCT intention-to-treat analysis was used. This approach ensures that participants are analysed according to their original allocation. However, of the included participants 90% and 84% completed the intervention and follow up, respectively, which may be considered as high proportion of completers. In addition, there were no differences in the number of drop-outs between the groups; therefore it was assumed that the missing data was completely random.

5.3.1.1.3 The intervention

A fact that may have contributed to the observed null results on the IHD risk factors was the relatively low intensity of the intervention (provision of dietary guidelines to the study participant through leaflets and a website), that may have been too low to ensure the necessary adherence in the two intervention groups that would allow detection of the expected effects of the dietary guidelines on IHD risk factors. In contrast, other studies which did find effects of the dietary guidelines on cardiometabolic risk factors included higher intensity intervention measures like food provisioning and dietary advice through face-to-face meetings with dietitians [105, 106, 120].

5.3.1.1.4 Statistic, analysis and confounding

In the statistical analyses of papers II and III, two multiple liner regression models were applied to consider known or potential confounding factors which strengthens the interpretability of the results. Furthermore, interactions were tested to investigate whether the intervention had different effects between sexes, age groups, and BMI. However, confounding from unknown or unmeasured factors cannot be excluded.

A general strength of this thesis research is the availability of detailed dietary intake data collected using a web-based 7-day dietary record [37, 110]. Also, validation of the dietary method used and evidence for compliance with the intervention were provided through an objective biomarker of intake. In addition, sub-analysis excluding under- and over-reporters was also applied, which made it possible to consider the potential information-bias of under- and over-reporting in the self-reported dietary data.

5.3.1.2 External validity

Some issues regarding generalisability of the results should be considered.

First of all, the criteria for inclusion were that the participants should have a minimum of one self-reported risk factor for IHD. However, although the majority of the participants were overweight or obese and had an elevated waist circumference, the study participants were generally healthy, and in that regard comparable with the general Danish population. A newly published report found that 51% and 16.8 % of the Danish population is overweight or obese [(38)], comparable with the proportion of overweight and obese study participants of the DIPI RCT.

A second issue regarding generalisability is that the study participants resided in areas of Greater Copenhagen with a higher level of education compared with the general Danish population [(37)]. It is well known that higher educational levels is associated with a healthier lifestyle and as such it may be more challenging to bring about dietary changes in populations with lower educational levels who may be assumed to be less health conscious than the study participants [(38)].

5.4 Dietary assessment method and objective markers for intake

Availability of an appropriate and valid method for assessing dietary intake is an essential component in the investigation of diet and disease. The method used in any study should therefore be based on the foods or nutrients of interest and the capacity of the target group to provide the needed details.

Long-term food recording methods like the FFQ, which is widely used in observation studies, rely on recall by the respondent and inherently gives rise to measurements errors but are simple and inexpensive to use, and the burden on the respondent is low.

In contrast to the FFQ, the dietary recording method used in the research of the present thesis does not depend on the respondent's memory and allows for collection of more detailed information about foods. However, both the cost and burden on the respondent are much higher than with the FFQ. Therefore, we only collected dietary intake on three occasions in the DIPI RCT: at baseline, after 6 months of intervention, and again after another 6 months of follow-up.

When investigating changes in dietary intake over a period of 6 months, it is difficult to detect information on changes in the usual intake of the specific foods and nutrients included in the provided dietary guidelines. Repeatedly recording the dietary intake during the intervention and the

follow-up periods would have been too big of a burden to respondents and might have caused undesirable dropouts.

Future development of dietary assessment methods to accurately measure dietary intake over a period of time is therefore crucial and these should include repeated measurements of dietary intake and use of multiple biomarkers for intake.

In the present thesis research, estimated whole grain intake was validated against alkylresorcinol as an objective biomarker. Alkylresorcinol was chosen as a biomarker because of the high relevance of whole grains to human health. The results showed that self-reported whole grain intake was associated with plasma alkylresorcinol concentrations at baseline. Furthermore, from cross-classification between reported whole grain intake and alkylresorcinol concentrations, it was found that 70% of participants were classified in the same quartile or an adjacent quartile, which is in agreement with findings from studies by Ross et al. [145] and Biltoft-Jensen et al. [112].

It would have been interesting to include more biomarkers for further validation of dietary intake such as n-3 fatty acids, using phospholipids as a marker for usual fish intake [146] and serum carotenoids as a biomarker for fruit and vegetable intake [147]. However, due to the prohibitively high cost of biomarker analyses, this was not possible.

5.5 Implications of the research results

In paper I the use of a dietary index approach enabled us to describe associations between food and nutrient intake in individuals with dietary patterns that were more or less compatible with the current Danish official dietary guidelines and cardiometabolic risk factors. Overall, the cross-sectional study of paper I adds to the growing body of evidence showing that adherence to different national dietary guidelines and diets that are rich in fruit and vegetables, whole grains, and fish and low in SFA and sweets are associated with a better cardiometabolic risk profile. However, the results of the present study need to be further tested and validated in both larger-scale observational studies with sufficient power and eventually in RCTs to enable conclusions to be made about cause and effect relationships.

Along those lines, it is important to further investigate the inter-relationship between diet quality, IHD risk factors, and BMI. In paper I, when further adjustment for BMI was made, the observed inverse association between the DQI score and LDL/HDL ratio, TAG, and trunk fat was attenuated

and no longer statistically significant, which strengthens the importance of BMI as a factor in cardiovascular disease prevention. Therefore, one might question whether the association of closer adherence to Danish official dietary guidelines and a more beneficial cardiometabolic risk profile is due to the effect of the official Danish guidelines, expressed in the DQI, on BMI and body composition including trunk fat, or whether the association is independent. The results of this study further strengthen the need to conduct sex-stratified analyses in relation to investigation of primary prevention of IHD risk.

In paper II, we found an increased intake of some of the recommend foods and nutrients included in both sets of dietary guidelines. This was supported by supplementary analysis of a short- and long-term increased DQI score, indicating increased adherence to both sets of dietary guidelines. As mentioned earlier, the purpose of the DQI originally developed by Knudsen et al. was to evaluate the overall diet quality based on the Danish official dietary guidelines in 2005 and not the potential health effects associated with the included foods and nutrients, the aim of paper I [91]. However, although the two sets of dietary guidelines investigated in the present study differ in terms of numbers and wording, the DQI still captures the essential elements of the targeted substitution dietary guidelines and can be used to indicate a change in adherence to both the targeted substitution dietary guidelines in this trial and the Danish official dietary guidelines and.

The results of this thesis research demonstrate that by simply providing advice about dietary guidelines using leaflets and a website it is possible to impose some dietary changes in a population of adults. However, the intensity of the intervention (leaflets, a website including information on dietary guidelines and recipes, and biweekly e-mails with new recipes) may have been too weak, which may have contributed to the null results on the IHD risk factors.

6 Conclusion

In conclusion, closer adherence to the current Danish official dietary guidelines was associated with a more beneficial cardiometabolic risk profile in a Danish adult population with at least one self-reported risk factor for IHD. However, the results highlight the need to conduct sex-stratified analyses on IHD risk in this particular population.

The results of the DIPI RCT showed that short-term, after 6 months of intervention, the targeted substitution dietary guidelines were more effective than the Danish official dietary guidelines in changing the diet, resulting in a dietary composition of the overall diet that was more cardioprotective compared with the habitual diet. However, after an additional 6 months of follow-up, the long-term effects of the two sets of dietary guidelines were similar, when compared with the habitual diet. This was supported by the observed positive changes in DQI score, indicating an increased adherence to both sets of dietary guidelines throughout the intervention and follow-up periods, compared with the habitual diet.

However, neither the targeted substitution dietary guidelines nor the Danish official dietary guidelines showed any overall short- or long-term effects on any of the included risk factors for IHD compared with the habitual diet.

7 Perspective

As a worldwide leading cause of morbidity and mortality, IHD is a primary consideration in the development of evidence-based dietary guidelines. Enhancing the understanding and knowledge of the association between nutrition and health will continue to progress, as the optimal strategy for prevention of IHD through diet is still far from being defined. The findings of the three papers included in this thesis will add to this knowledge and will be of importance in the revision of future dietary guidelines and prevention of IHD.

However, the present results also demonstrate the challenges in promoting dietary guidelines that—when followed—have the potential to reduce the risk of IHD among the general population in an effective way. Behavioural changes are preferred over pharmacological or surgical interventions as these could lead to adverse effects, but implementing and ensuring adherence to the former are more challenging.

Future studies could therefore focus on addressing the need for innovative ways to promote and ensure adherence to dietary guidelines. In line with this, many new initiatives using computer or mobile phone technologies have been developed to promote healthy lifestyles, and how use of these new technologies may help in improving adherence to dietary guidelines is an important topic of future research.

Human behaviour is influenced at many different levels. In some dietary RCTs, a beneficial change in IHD risk factors was observed in participants who were provided with foods free of charge. In addition, considering the missing results on IHD morbidity and mortality of many multiple risk factor intervention studies, another approach could be to focus on the effects of different structural initiatives such as encouraging the consumption of healthy foods by imposing additional taxes on unhealthy foods and legislation, for example, to alter the fat content of foods and better labelling, all of which could help individuals to adopt healthier dietary patterns and could potentially lead to prevention of IHD. Such structural initiatives could target the entire population, including children and adolescents.

Future research could therefore be focused on the complexity of human behaviour in relation to imposing changes to dietary patterns, including behavioural theories, and the effects of structural initiatives like promotion of healthy foods and introduction of taxes and legislation to advocate healthy dietary patterns.

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9 Appendices

Appendix A: Paper I to III

Paper I: Associations between adherence to the Danish Food-Based Dietary Guidelines and cardiometabolic risk factors in a Danish adult population: the DIPI study

Paper II: Short and long term dietary effects of applying Substitution dietary guidelines and Official dietary guidelines in a Danish adult population: The DIPI randomised controlled trial.

Paper III: Effects of targeted substitution dietary guidelines on ischemic heart disease risk factors in an adult Danish population: The DIPI randomised controlled trial.

Paper I:

Associations between adherence to the Danish Food-Based Dietary Guidelines and cardiometabolic risk factors in a Danish adult population: the DIPI study

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Associations between adherence to the Danish Food-Based Dietary Guidelines and cardiometabolic risk factors in a Danish adult population: the DIPI study

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Abstract

Diet is recognised as one modifiable lifestyle factor for ischaemic heart disease (IHD). We aimed at investigating the associations between adherence to the Danish Food-Based Dietary Guidelines (FBDG) indicated by a Dietary Quality Index (DQI) and selected cardiometabolic risk factors in a cross-sectional study with 219 Danish adult participants (59 %women; age 31–65years) with a minimum of one self-rated risk marker of IHD. Information regarding diet was obtained using web-based dietary assessment software and adherence to the Danish FBDG was expressed by a DQI calculated from 5 food and nutrient indicators (whole grain, fish, fruit and vegetables, energy from saturated fat and from added sugar). Background information, blood samples and anthropometrics were collected and blood pressure was measured. Linear regression analyses were used to evaluate the association between DQI and cardiometabolic risk factors. DQI was inversely associated with LDL:HDL ratio and TAG (−0·089 per unit; 95 % CI −0·177, −0·002 and −5 % per unit; 95 % CI −9, 0, respectively) and positively associated with HDL-cholesterol (0·047 mmol/l per unit; 95 % CI 0·007, 0·088). For men, DQI was inversely associated with BMI (−3 %per unit; 95 % CI −5, −1), trunk fat (−1 % per unit; 95 % CI −2, −1), high-sensitivity C-reactive protein (−30 % per unit; 95 % CI −41, −16 %), HbA_{1c} (−0·09 % per unit; 95 % CI −0·14, −0·04), insulin (−13 % per unit; 95 % CI −19, −7) and homoeostatic model assessment-insulin resistance (−14 % per unit; 95 % CI −21, −7). In women, DQI was positively associated with systolic blood pressure (2·6 mmHg per unit; 95 % CI 0·6, 4·6). In conclusion, higher adherence to the current Danish FBDG was associated with a more beneficial cardiometabolic risk profile in a Danish adult population with a minimum of one self-rated risk factor for IHD.

Key words: **Dietary patterns: Diet quality: Diet index: Cardiovascular risk factors: Cross-sectional studies**

Ischaemic heart disease (IHD) is one of the major causes of morbidity and mortality worldwide[1,2]. Diet is recognised as one of several modifiable lifestyle factors for the prevention of IHD[1,3,4].

During the past decades, research on diet–disease associations has focused on measurements of overall quality of diets and dietary patterns as opposed to the traditional approach in dietary research with focus on single nutrients and foods[5–8]. This change in research focus is justified by the notion that people eat composite diets and meals with nutrients and foods in combination.

Several dietary scores and dietary quality indices have been developed to assess adherence to different healthy food patterns and national Food-Based Dietary Guidelines (FBDG)[6,9,10]. Some of the most commonly used are The Mediterranean diet score indicating compliance with the traditional dietary pattern followed by Mediterranean populations, and the American Healthy Eating Index (HEI), which assesses adherence with the Dietary Guidelines for Americans[11]. Both observational and intervention studies have shown a protective effect on the development and mortality of CVD with a higher compliance to the Mediterranean diet and the Dietary Guidelines for Americans[12–16].

The Mediterranean diet score and the American HEI are considered most suitable for the Mediterranean countries and the Americans and for countries with similar food cultures,

Abbreviations: BP, blood pressure; DQI, Dietary Quality Index; DQS, Dietary Quality Score; E%, energy contribution; FBDG, Food-Based Dietary Guidelines; HEI, Healthy Eating Index; HOMA-IR, homoeostatic model of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IHD, ischaemic heart disease; OR, over-reporters; UR, under-reporters; WC, waist circumference.

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respectively. In the Nordic countries, including Denmark, a different food culture exists with a dietary pattern relatively abundant in certain fruit and vegetables (especially berries, cabbages, root vegetables and legumes), potatoes, whole-grain cereals, dairy and meat products[17]. The Danish food culture and food preferences were included as an integrated part in the development of the current Danish FBDG when translating the scientific evidence regarding the association between diet and risk of diseases into quantified FBDG[18].

In Denmark, two dietary quality indices have been developed to measure adherence to the Danish FBDG from 2005[19,20]. One is the Dietary Quality Score (DQS), which is based on a forty-eight-item FFQ, and uses a three-point scoring system for each of four food groups: fish, fruit, vegetables and fats. The DQS has been found to be inversely associated with serum lipids, homocysteine and absolute risk of IHD in men and women aged 30–60 years[19]. The other, the Diet Quality Index (DQI), is based on dietary data from a 7-d pre-coded food diary, and uses a sum of six scores of food and nutrients based on the 2005 FBDG relating to dietary intake[20,21]. In continuation of the update of the Danish FBDG in 2013, an updated version of the DQI was applied to reflect the changes in the FBDG[18,22]. The updated DQI is based on five food and nutrient indicators, including whole grain, fish, fruit and vegetables and energy % from saturated fat and from added sugar[20,22].

The objective of this study was to investigate associations between adherence to the current Danish FBDG assessed by a DQI and selected cardiometabolic risk factors in a Danish adult population with a minimum of one self-rated risk factor of IHD.

Methods

Study design

The study was based on baseline data from the study Diet and Prevention of Ischemic Heart Disease – a Translational Approach (DIPI) (www.DIPI.dk), which included a 6-month randomised, single-blinded parallel, dietary intervention study in a real-life setting, with a 6-month follow-up. The study was designed to assess the effects of dietary substitution guidelines specifically aimed at the prevention of IHD on dietary intake and IHD risk factors in the general adult Danish population. This paper reports on the baseline cross-sectional data.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by The Capital Region of Denmark Ethics Committee (Journal no. H-1-2013-110) and by the Danish Data Protection Agency (Journal no. 2013-54-0571). Written informed consent was obtained from all study participants, and they received a small remuneration of about 34 GBP for their participation in the study. The study was registered at ClinicalTrials.gov (registry name 'DIPI', ID no. NCT02062424).

Study participants

Potential participants were identified using a unique personal identification number assigned to all Danish citizens in the Civil Registration System[23]. In total, 5000 men and women born in 1949–1984 and living in a defined area of the greater Copenhagen were invited by letter to participate in the study. The number of invited participants was based on previous experience of a low response rate when recruiting participants for long-term interventions. Overall, 334 responded on the invitation and were thus screened from a self-administered questionnaire including questions on the inclusion and exclusion criteria. The potential participants were asked in the questionnaire to measure and report their height in metres, weight in kg, their waist circumference (WC) 2 cm above their belly button and whether or not they were physically active for more than 15 min/week. Furthermore, the self-administered questionnaire included questions on the exclusion criteria; see below. After screening, the eligible participants were invited to an information meeting, which included an introduction to the web-based dietary assessment software. Of the eligible participants who participated in the information meeting, 100 % agreed to participate and provided informed consent.

The inclusion criteria were age between 30 and 65 years, and a minimum of one self-rated risk factor of IHD – that is overweight or obesity (BMI $\geq$ 25) – WC $\geq$80 cm for women and $\geq$94 cm for men, and/or physical inactivity defined as being moderately physically active in leisure time for 15 min or less per week.

The exclusion criteria were current smoking, pregnancy or plans to become pregnant within the next 12 months, breast-feeding, history of CVD, type 2 diabetes, chronic disease/disorders that could affect the results of the study (the chronic diseases that the subjects reported were evaluated by the clinical physician in charge), drug abuse within the past 12 months, regular alcohol consumption >21 units/week for men or >14 units/week for women, allergies or intolerance of the food groups included in the dietary guidelines, consumption of dietary supplements with high doses of nutrients that could have a potential effect on IHD risk factors (e.g. fish oils) and/or no access to a computer and internet.

Measures

Dietary intake and calculation of diet quality index. The study participants recorded their dietary intake using a web-based dietary assessment software for 7 consecutive days[24]. The web-based dietary assessment software was originally developed and validated for children aged 8–11 years and slightly customised to fit the adult study population of the DIPI study[24,25]. At least 4 d of food reporting had to be completed by the study participant for inclusion of the study participants in the analysis[21].

The dietary assessment software was structured according to a typical Danish meal pattern covering breakfast, lunch, dinner and three in-between meals. The participants could estimate the amount consumed by selecting the closest portion size among four different digital images in eighty photograph series. Internal checks for frequently forgotten foods (spreads, sugar, sauces, dressings, snacks, candy and beverages) were included. Furthermore, the participants reported the intake of nutritional supplements and whether a day represented usual or unusual intake, including reasons for unusual intakes such as illness. If a participant failed to report for a day, the participant was reminded by an email the next day[24].

Intakes of food items, energy and nutrients were calculated for each study participant as an average of 7 d using the software system General Intake Estimation System (GIES) version 1.000.i6 (National Food Institute, Technical University of Denmark) and the Danish Food Composition Databank version 7.0 (National Food Institute Technical University of Denmark, 2009).

Adherence to the Danish FBDG was evaluated based on a DQI published earlier and updated to the current Danish FBDG[20,22], including intake of whole grain (min 75 g/10 MJ per d), intake of fish (min 350 g/10 MJ per week), intake of fruit and vegetables (min 600 g/10 MJ per d), energy from saturated fat (max 10 E%) and energy from added sugar (max 10 E%). The DQI was based on intake adjusted to 10 MJ, as this is the unit for the FBDG[18].

A DQI for each study participant was calculated – adapted from[20] – as the ratio of the actual intake and the recommend intake of each of the five guidelines included in the index. For example, if a study participant had an intake of 60 g/10 MJ per d whole grain, the score was 60/75 = 0·8. For the included guidelines with an upper limit of a recommended intake, the DQI was calculated as 1 − ((intake − recommended)/recommended), and thus for a study participant with an intake of 13 % energy from added sugar the DQI was calculated as 1 − ((13–10)/10) = 0·7.

In contrast to the original DQI, we did not have a maximum score in individuals with an intake exceeding the cut-off values[20]. The total score was calculated as the sum of the five scores, a higher score meaning a higher degree of compliance with the FBDG.

Under- and over-reporters. Under- and over-reported energy intake (EI) was defined as a ratio of reported mean EI:BMR and classified by cut-offs suggested by Black[26,27]. Under-reporters (UR) were defined as EI:BMR ≤ 1·05 and over-reporters (OR) were defined as EI:BMR ≥ 2·28, using a physical activity level of 1·55 (data not shown).

Assessment of cardiometabolic risk factors

Blood samples. Fasting blood samples from venepuncture were analysed for concentrations of TAG, total cholesterol, HDL-cholesterol, high-sensitivity C-reactive protein (hsCRP), glucose, HbA_{1c} and insulin. The blood samples were collected and handled according to the hospital routines. TAG, total cholesterol, HDL-cholesterol and glucose were measured in plasma by Reflection Spectroscopy at 540 nm and hsCRP was measured in plasma by Reflection Spectroscopy at 660 nm (Apparatus Vitros 5.1 FS; Ortho-Clinical Diagnostics). HbA_{1c} was measured in plasma with HPLC (D-100; Bio-Rad). Fasting plasma insulin was measured using the sandwich ELISA analysis principle (ADVIA Centaur XP; Siemens). VLDL-cholesterol was calculated from TAG, using the equation plasma VLDL-cholesterol = plasma TAG × 0·45, and LDL-cholesterol was calculated using the Friedewald equation[28].

The homoeostatic model assessment (HOMA) was used to estimate insulin resistance (HOMA-IR). HOMA-IR was calculated using the formula HOMA-IR = (glucose (nmol/l) × insulin (mU/ml)/22·5), using fasting values[29].

Anthropometric measurements (height, weight and waist circumference). Height was measured to the nearest 0·5 cm, on a wall-mounted stadiometer (SECA). Body weight was measured in kg and trunk fat was registered on a fat analysis weight (Tanita BC 418 MA). The subjects had to be fasting. Waist and hip circumference was measured twice, with an anthropometric tape (SECA 201), and the average was reported.

BMI was defined as weight in kg divided by squared height in metres (kg/m^2).

Blood pressure and heart rate. Seated blood pressure (BP) and heart rate (HR) were measured in duplicate after 5 min of rest in the subjects' left arm, using an electric sphygmomanometer according to standardised procedures. The subjects had to empty their bladder before the measurement and were not allowed to converse during the measurement, nor have their legs crossed. If the diastolic BP differed more than 5 mmHg, further measurements were done, until at least in two measurements the diastolic BP differed ≤5 mmHg. The average value of the two BP and HR measurements was calculated.

Assessment of background questionnaires. Lifestyle questionnaires were used to obtain information about the participant's education level (primary school/high school, associate degree, under-graduate, graduate) and the level of physical activity at leisure time (extremely active, moderately active, sedentary or inactive). The question about the level of physical activity was based on one question about the study participants' physical activity during leisure time in the past 6 month and was based upon the Danish National Health Profile[30].

Statistical analysis

For a parallel design, statistical power calculations based on evidence from previous similar studies[31–33] were used to estimate that sixty-two subjects in each intervention arm were sufficient to detect a difference of 0·25 mmol/l LDL-cholesterol (SD 0·49) ($\alpha = 0{\cdot}05$, $\beta = 0{\cdot}8$). To allow for a drop-out of 20 %, the number of participants was set to a total of 225. Self-rated weight (kg), WC and BMI from the screening self-administered questionnaire were compared with weight, WC and BMI measured at baseline by a paired *t* test. Baseline characteristics and dietary intake of the study participants were summarised for men and women using medians and 80 % central range for continuous variables and proportions for categorical variables.

Linear regression analyses were used to evaluate the association between DQI and cardiometabolic risk factors. Three models were applied; a simple model adjusted for sex and age (<50 or ≥ 50) (model 1a), a multivariate model further adjusted for education (primary school/high school, associate degree, under-graduate or graduate) and physical activity at leisure time (extremely active, moderately active, sedentary or inactive) (model 1b), and a final multivariate model adjusted as model 1b plus BMI (model 2). Furthermore, sensitivity analysis excluding UR and OR was made to investigate the impact of UR and OR on the associations between DQI and cardiometabolic risk factors.

All the models were tested for statistically significant interactions between DQI and sex and DQI and age. These interactions were tested to investigate whether the associations were different for men and women and for participants <50 years of age or 50 years of age or above. If an interaction was significant, the DQI estimates for men and women and DQI estimates by age were given. To check the model assumptions, the standardised residuals of the final models were examined for normality, variance homogeneity and linearity. BMI, waist:hip-ratio, hsCRP, VLDL-cholesterol, TAG, glucose, insulin and HOMA-IR were all logarithm10 transformed to normalise the distribution and to improve variance homogeneity. For the above-mentioned variables, which were log-transformed, the estimates and 95 % CI are presented as percent difference.

The statistical analyses were carried out using RStudio (version 0.99.441 – © 2009–2015; RStudio, Inc.). Statistical significance was established at $P < 0{\cdot}05$.

Results

Baseline characteristics of study participants

A total of 222 participants met the inclusion and exclusion criteria and were enrolled into the study, which was 67 % of the initially screened potential study participants. Significant differences ($P < 0{\cdot}05$) in weight and WC were found between self-reported and measured at baseline. The participants' self-reported weight and WC were higher than the measured weight and WC at baseline. However, no difference in BMI was found. In all, 17 % of the women and 7 % of the men did not meet the inclusion criteria when measured at baseline (data not shown). For three participants, a full baseline examination was missing, and they were therefore excluded. Of the remaining 219 study participants, those who were taking cholesterol-lowering (n 5) and/or BP-lowering (n 3) medications were excluded from the statistical analysis of the association between DQI and lipid biomarkers and DQI and BP, respectively. In addition, biochemical analysis of hsCRP was not possible in fifteen study participants; therefore, for the statistical analysis of the association between DQI and hsCRP, the number of participants was 204. Furthermore, it was not possible to get sufficient blood from one of the study participants for the biochemical analysis of the glycaemic biomarkers, and thus for the statistical analysis of the association between DQI and glycaemic biomarkers (n 218).

Baseline characteristics of the 219 study participants included in the statistical analysis are presented in Table 1. Of the 219 study participants, ninety were men and 129 were women. The median age of the study participants was 51 years (10th–90th percentile (p10–p90) 37–61).

Diet quality index, total energy intake and dietary composition

For the whole study population the median DQI was 4·5 (p10–p90 3·0–6·5). The median DQI score was higher for women than for men. Furthermore, the median individual DQI score for added sugar was higher than the median DQI scores for whole grain, fish, fruit and vegetables and saturated fat. Median total EI for men was 10·1 MJ (p10–p90 7·2–13·7) and for women it was 7·8 MJ (p10–p90 5·2–10·5) (Table 2). Medians (p10–p90) of the Diet Quality Index score (DQI), energy (MJ/d), diet composition (g/10 MJ per d) and energy contribution (E%) of macronutrients and dietary fibre (g/MJ) of the study population divided in tertiles of the DQI are presented in the online Supplementary Table S1.

Association between Dietary Quality Index and cardiometabolic risk factors

Table 3 summarises the associations between DQI and cardiometabolic risk factors in the study population. In the following section, only results from model 1b will be presented.

We found an inverse association between DQI and the lipid risk factors – LDL:HDL ratio and TAG (−0·089 per unit DQI; 95 % CI −0·177, −0·002 and −5 % per unit DQI; 95 % CI −9, 0, respectively) – and a positive association between DQI and HDL-cholesterol (0·047 mmol/l per unit DQI; 95 % CI 0·007, 0·088). For men only, we found an inverse association between DQI and BMI (−3 % per unit DQI; 95 % CI −5, −1), trunk fat (−1 % per unit DQI; 95 % CI −2, −1), hsCRP (−30 % per unit DQI; 95 % CI −41, −16) and the glycaemic risk factors, HbA_{1c} (−0·09 % per unit DQI; 95 % CI −0·14, −0·04), insulin (−13 % per unit DQI; 95 % CI −19, −7) and HOMA-IR (−14 % per unit DQI; 95 % CI −21, −7). Furthermore, we found a positive association between DQI and systolic BP in women (2·6 mmHg per unit DQI; 95 % CI 0·6, 4·6).

Under- and over-reporters

Overall, 21 % of the participants were classified as UR and 1 % as OR. Of those classified as UR, 89 % were overweight or obese, 46 % of the UR were men and all of the OR were men.

The associations between DQI and most of the variables included were the same whether UR and OR were included in the analysis or not. However, for the metabolic markers BMI, trunk fat, systolic BP and hsCRP, the interaction between DQI and sex was no longer significant when excluding UR and OR. Except for BMI, the association between DQI and these metabolic markers was now significant for the whole study population (trunk fat: −1 % per unit DQI; 95 % CI −2, −0·4, systolic BP: 1·7 mmHg per unit DQI; 95 % CI 0·08, 3·36, and hsCRP −23 % per unit DQI; 95 % CI −32, −12). Furthermore, the metabolic markers waist:hip-ratio changed from being non-significant to significant (−1 per unit DQI; 95 % CI −2, 0). In addition, the observed positive association between DQI and HDL-cholesterol was only significant in study participants aged 50 years or above when excluding UR and OR from the analysis (0·09 mmol/L per unit DQI; 95 % CI 0·034, 0·045).

Discussion

In the present study, a higher adherence to the current Danish FBDG, assessed by a DQI, was associated with a more beneficial cardiometabolic risk profile in a Danish adult population with a minimum of one self-rated risk factor for IHD. The main findings were the inverse associations between the DQI and the lipid risk factors: LDL:HDL ratio and TAG and the positive

Table 1. Baseline characteristics of the study participants divided by sex (Medians and 10th–90th percentiles (p10–p90); percentages and numbers)

	All (*n* 219)		Men (*n* 90)		Women (*n* 129)	
Participant characteristics	Median	p10–p90	Median	p10–p90	Median	p10–p90
Age (years)	51·0	37–61	50·5	36–61	51·0	37–61
Metabolic markers						
Weight (kg)	82·6	65·3–103·6	88·4	79–117·5	74·6	62·3–92·8
BMI (kg/m^2)*	26·7	22·9–33·6	27·0	23·9–33·9	26·3	22·6–32·9
Weight status*						
Normal weight						
%	27		16		36	
n	60		14		46	
Overweight						
%	53		66		43	
n	116		60		56	
Obese						
%	20		18		21	
n	43		16		27	
Waist circumference (cm)	92·4	79·8–105·2	97·4	90·8–118·3	87·0	76·8–99·5
Hip circumference (cm)	107·1	99·4–120·0	106·7	102·3–118·7	108·2	98·9–120·4
Systolic BP (mmHg)†	130	110–154	135	119–164	125	108–149
Diastolic BP (mmHg)†	80	69–94	82	72–98	78	68–92
hsCRP (mg/l)‡	1·4	0·2–6·3	1·0	0·2–5·4	1·9	0·2–8·3
Lipid biomarkers§						
Total cholesterol (mmol/l)	5·3	4·1–6·8	5·5	4·4–6·9	5·2	4·0–4·5
LDL-cholesterol (mmol/l)	3·2	2·2–4·5	3·5	2·5–4·7	3·1	2·1–4·5
HDL-cholesterol (mmol/l)	1·4	1·0–3·9	1·2	0·9–1·9	1·6	1·1–2·3
LDL:HDL ratio	2·3	4·1–6·8	2·8	1·7–4·3	2·0	1·2–3·0
VLDL-cholesterol (mmol/l)	0·5	0·3–1·0	0·6	0·3–1·2	0·4	0·3–0·8
TAG (mmol/l)	1·1	0·6–2·3	1·3	0·7–2·6	0·9	0·6–1·8
Glycaemic biomarkers‖						
Glucose (mmol/l)	5·5	5·0–6·3	5·6	5·1–6·4	5·4	4·9–6·1
HbA_{1c} (%)	5·0	4·5–5·5	5·0	4·6–5·5	5·0	4·5–5·5
Insulin (pmol/l)	58	30–100	59	31–127	58	30–94
HOMA-IR	2·0	1·0–3·8	2·2	1·0–4·6	2·0	1·0–3·6
Educational level						
Primary school or high school						
%	25		29		23	
n	55		26		30	
Associate degree						
%	8		6		10	
n	18		5		13	
Under-graduate school						
%	40		31		46	
n	87		28		59	
Graduate school						
%	27		34		21	
n	58		31		27	

BP, blood pressure; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homoeostatic model of insulin resistance.
* BMI is calculated as weight in kg divided by the square of height in m (kg/m^2). 18·5–25 kg/m^2: normal weight, 25–30 kg/m^2: overweight, >30 kg/m^2: obese.
† All; 216, men; eighty-nine, women; 127, after exclusion of those using BP-lowering medication.
‡ All; 204, men; eighty-seven, women; 117, due to lack of hsCRP in biochemical analyses.
§ All; 214, men; eighty-five, after exclusion of those using cholesterol-lowering medication.
‖ All; 218, women; 128, as it was not possible to draw enough blood to the glycaemic biomarker analysis from one of the female study participants.

association with HDL-cholesterol. For the male study participants, an inverse association between DQI and BMI, trunk fat, hsCRP and the glycaemic risk factors, HbA_{1c}, insulin and HOMA-IR was observed. Furthermore, we found a positive association between DQI and systolic BP in women.

When we controlled for the effect of BMI on cardiometabolic risk factors, the associations between DQI and trunk fat, LDL:HDL ratio and TAG were attenuated and no longer statistically significant, suggesting that BMI could be an important factor in CVD prevention. Of that notion it is important to further investigate whether the association of higher adherence to the Danish FBDG and a more beneficial cardiometabolic risk profile is due to the effect of the Danish FBDG, expressed by DQI, on BMI and body composition, including trunk fat, or whether the association is independent. We only found an inverse significant association between DQI and BMI in men, suggesting that a higher DQI score (indication of a higher adherence to the Danish FBDG) is only associated with a lower BMI in men and not women. The fact that DQI and BMI was only associated in men and not in women could be explained by the higher number of normal-weight women than men, and the higher number of overweight men than women. Moreover,

Table 2. Overall Diet Quality Index (DQI) score and DQI scores for individual components, energy (MJ/d), diet composition (g/10 MJ per d) and energy contribution (E%) of macronutrients and dietary fibre (g/MJ) of the study participants
(Medians and 10th–90th percentiles (p10–p90))

Participants	Guidelines and recommendations*	All (219)		Men (*n* 90)		Women (*n* 129)		DQI score	
		Median	p10–p90	Median	p10–p90	Median	p10–p90	Median	p10–p90
Overall DQI		4·5	3·0–6·5	4·2	2·7–6·2	4·6	3·0–6·6		
Energy (MJ/d)		8·6	5·8–12·4	10·1	7·2–13·7	7·8	5·2–10·5		
Diet composition (g/10 MJ/d)									
Bread and cereals*		218	141–301	215	149–291	220	136–307		
Whole grains	75 g/d	61	31–111	56	30–117	66	34–108	0·8	0·4–1·5
Meat and meat products	<70 g/d	139	59–249	166	85–282	124	45–224		
Fish and fish products†	50 g/d	46	5–111	41	5–109	48	5–125	0·9	0·1–2·2
Poultry and poultry products‡		32	0–102	31	0 –103	32	0–99		
Fruit and vegetables	600 g/d§	388	177–690	324	144–508	452	243–749	0·6	0·3–1·2
Fruit and fruit products		143	43–331	100	23–245	180	64–362		
Vegetables and vegetable products		220	110–425	194	84–348	237	132–469		
Vegetables, coarse‖		97	29–208	83	23–191	104	39–215		
Vegetables, fine‖		114	49–228	95	37–167	122	68–255		
Potatoes and potato products		57	7–138	73	15–152	47	2–126		
Milk and milk products		243	68–513	213	68–484	272	70–531		
Cheese and cheese products	Choose low fat	45	11–129	36	5–97	50	16–156		
Edible fats		31	18–46	30	16–48	31	18–45		
Sugar and candy	Reduce intake	35	10–77	31	10–76	38	14–77		
Energy distribution									
Protein (E%)	10–20	17	14–22	17	14–21	17	14–22		
Fat (E%)	25–40	35	29–42	35	28–40	36	29–43		
SFA (E%)	<10	13	11–17	13	10–17	13	11–16	0·7	0·4–0·9
MUFA (E%)	10–20	13	10–17	13	10–16	14	11–18		
PUFA (E%)	5–10	5	4–7	5	4–6	5	4–7		
Carbohydrate (E%)	45–60	44	35–53	43	34–53	45	35–53		
Added sugar (E%)	<10	7	2–13	7	2–15	7	3–13	1·3	0·7–1·8
Dietary fibre (g/MJ)	> 3 g/MJ	2	2–3	2	2–3	3	2–4		
Alcohol (E%)¶	<5	5	1–14	7	1–16	4	0–10		

* Guidelines and recommendations according to the official Danish Food-Based Dietary Guidelines[18] and the Nordic Nutrition Recommendations, 2012[8].
† All; *n* 201, men; eighty-three, women; 118 after exclusion of those who did not eat fish.
‡ All; 180, men; seventy-six, women; 104 after exclusion of those who did not eat poultry.
§ Eat 6 a day – equivalent to about 600 g of vegetables and fruit. At least half should be vegetables.
‖ Vegetables are classified from type of food groups (e.g. all types of cabbage, root vegetables and onions are classified as coarse vegetables and all vegetables with a high water content like tomatoes and salad are classified as fine vegetables).
¶ All; 194, men; eighty-one, women; 113 after exclusion of those who did not drink alcohol.

Table 3. Linear regression of the associations between adherence to the Danish Food-Based Dietary Guidelines (FBDG) assessed by a diet quality index (DQI) and cardiometabolic risk factors in a Danish adult population with minimum one self-rated risk factor for Ischaemic heart disease (*β*-Coefficients per unit DQI and 95 % confidence intervals)

	Model 1a†		Model 1b‡		Model 2§	
	β	95 % CI	*β*	95 % CI	*β*	95 % CI
Metabolic markers (*n* 219)						
BMI (kg/m^2) (%)	0	−2, 1	0	−1, 2		
Men	−3*	−5, 1	−3**	−5, −1		
Women	0	−2, 1	0	−1, 2		
Waist:hip-ratio (%)	−1	−1, 0	−1	−1, 0	−1	−2, 0
Trunk fat (%)	−1*	−1, 0	0	−1, 1	0	−1, 0
Men			−1***	−2, −1		
Women			0·02	−0·69, 0·73		
Systolic BP (mmHg)‖	0·81	−0·7, 2·3	1·15	−0·36, 2·67	1·59*	0·11, 3·07
Men			−0·68	−3·04, 1·68		
Women			2·6*	0·63, 4·58		
Diastolic BP (mmHg)‖	−0·24	−1·19, 0·71	0·92	−0·3, 2·13	0·3	−0·56, 1·17
Men			−1·33	−2·78, 0·12		
Women			0·92	−0·3, 2·13		
hsCRP (mg/l) (%)¶	−16**	−25, −6	−16**	−25, −6	−12*	−21, −2
Men	−29***	−40, −15	−30***	−41, −16		
Women	−7	−19, 7	−5	−18, 9		
Lipid biomarkers (*n* 214)						
Total cholesterol (mmol/l)	0·005	−0·089, 0·099	−0·009	−0·104, 0·087	−0·006	−0·101, 0·090
LDL-cholesterol (mmol/l)	−0·017	−0·098, 0·065	−0·027	−0·110, 0·055	−0·024	−0·106, 0·059
HDL-cholesterol (mmol/l)	−0·052*	0·012, 0·092	0·047*	0·007, 0·088	−0·009	−0·071, 0·052
< 50 years					−0·009	−0·071, 0·052
> 50 years					0·071**	0·021, 0·120
LDL:HDL-ratio	−0·088*	−0·175, −0·002	−0·089*	−0·177, −0·002	−0·074	−0·158, 0·010
VLDL-cholesterol (mmol/l) (%)	−5*	−9, −1	−5	−9, 0	−4	−8, 0
TAG (mmol/l) (%)	−5*	−9, −1	−5*	−9, 0	−4	−8, 0
Glycaemic biomarkers (*n* 218)						
Glucose (mmol/l) (%)	0	−1, 1	0	−1, 1	0	−1, 1
HbA_{1c} (%)	0·02	−0·03, 0·06	0·02	−0·02, 0·06	0·02	−0·03, 0·05
Men	−0·09***	−0·13, −0·04	−0·09***	−0·14, −0·04	−0·07**	−0·12, −0·02
Women	0·02	−0·03, 0·06	0·02	−0·02, 0·06	0·02	−0·02, 0·05
Insulin (pmol/l) (%)	−4	−10, 2	−2	−7, 5	−3	−8, 3
Men (%)	−12***	−19, −6	−13***	−19, −7	−9**	−15, −3
Women (%)	−4	−10, 2	−7	−7, 5	−3	−8, 3
HOMA-IR (%)	−4	−10, 3	−1	−7, 6	−2	−8, 4
Men (%)	−13***	−20, −6	−14***	−21, −7	−10**	−16, −3
Women (%)	−4	−10, 3	−1	−7, 6	−2	−8, 4

BP, blood pressure; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homoeostatic model of insulin resistance.
*$P<0·05$, ** $P\leq0·01$, *** $P=0·001$.
† Simple linear regression model adjusted for sex and age.
‡ Multiple linear regression analysis adjusted for sex, age, education, physical activity at leisure time.
§ Multiple linear regression analysis adjusted for sex, age, education, physical activity at leisure time and BMI.
‖ *n* 216, after exclusion of those using BP-lowering medication.
¶ *n* 204 due to lack in biochemical analyses of hsCRP.

the sex-specific association between DQI and systolic BP and DQI and hsCRP was no longer present when we controlled for the effect of BMI. Here a positive association between DQI and Systolic BP and an inverse association between DQI and hsCRP were seen for the whole study population. Additionally, in the sensitivity analysis excluding UR and OR, a non-sex-specific significant association between DQI and trunk fat, systolic BP and hsCRP was observed. These results suggest that the observed sex-specific associations between DQI and the metabolic biomarkers BMI, trunk fat, systolic BP and hsCRP found in the analysis, including UR and OR, could be explained by dietary report errors. However, the results of the sensitivity analysis should be interpreted with caution, because of potential power issues when removing UR and OR from the analysis.

The inverse relationship between BMI and CRP is well recognised, and, moreover, it has been recognised that with increased adiposity CRP is further elevated[34]. The link between these risk factors is supported by the results of the present study, where an inverse association between DQI and BMI, trunk fat and hsCRP was found in men.

In addition, when controlling for the effect of BMI, the observed positive association between DQI and HDL-cholesterol was only significant in study participants aged 50 years or above, suggesting that BMI is a more important mediator for HDL-cholesterol levels for people less than 50 years of age. However, genetic studies have lately challenged the common concept of raising HDL-cholesterol as a marker for CVD risk reduction, and further studies are need to investigate the role of HDL-cholesterol in CVD prevention[35,36].

A main advantage of this study is the detailed assessment of the dietary data with the habitual diet measured during a 7-d consecutive dietary record using a validated method used for

the past two decades in the Danish National Survey of Dietary Habits and Physical Activity[21,37]. In addition, this study uses a DQI based on five food and nutrients from the Danish FBDG relevant for IHD risk, and thus a relatively easy way to measure adherence to the overall current FBDG. A limitation of this study is the observational design of the cross-sectional study, as this design does not allow conclusions on cause and effect relationships. Therefore, extrapolation of the results should only be made with caution as the study participants resided in areas of Greater Copenhagen with a relatively higher level of education compared with the general Danish population[37].

The sex-specific results with an inverse association in men only between DQI and the cardiometabolic risk factors BMI, trunk fat, hsCRP, HbA_{1c}, insulin and HOMA-IR are in line with two cohort studies using the HEI[38,39]. The cross-sectional study by Drewnowski *et al.*[39] including 5081 men and women, middle-aged French citizens with low CVD risk, investigated the association between the HEI and cardiovascular risk factors. Here an inverse association between the HEI and BMI was found in men only. Furthermore, Frazier-Woods *et al.* observed in another cross-sectional study containing 9797 adults men and women, with at least one CVD risk factor, that in men only the HEI score was inversely associated with insulin, HOMA-IR, HDL-cholesterol, TAG and CRP[38]. In contrast to our results, when Frazier-Wood *et al.* adjusted for BMI, the results were no longer significant. This strengthens the importance of BMI as an important factor in CVD prevention[38].

The sex-specific differences in the results in the present study could be accounted for by alcohol intake of those of the study participants who drank alcohol (*n* 194), as the men in the study drank more alcohol than the women. The median alcohol intake of the women was 3·9 E%, whereas the men had a median alcohol intake of 6·5 E%. It could be that men with a higher adherence to the Danish FBDG, expressed by a higher DQI score, also had a lower alcohol intake equivalent to the Danish recommendations of max seven drinks per week, corresponding to approximately 1 drink/d. A recent meta-analysis of 84 prospective cohort studies found that alcohol consumption of 2·5– 14·9 g/d (about ≤1 drink/d) was associated with a lower risk of cardiovascular mortality, compared with abstaining from alcohol[40]. This is supported by the findings of the present study, where we found that a higher DQI score was associated with a more beneficial cardiometabolic risk profile.

One other cross-sectional study by Toft *et al.* investigated the association between adherence to Danish FBDG, assessed by a DQS and CVD risk factors[19]. In this cross-sectional study including 6542 healthy men and women aged 30–60 years, it was found, in line with the findings of the present study, that higher adherence to the Danish FBDG 2005 was associated with a more beneficial CVD risk factor profile. In agreement with our results, Toft *et al.* also found that the DQS was inversely associated with TAG, but in contrast to our results the DQS was inversely associated with total cholesterol and LDL-cholesterol. However, Toft *et al.* did not find a positive association between the DQS and HDL-cholesterol like in the present study.

Furthermore, our results are supported by the findings of another cross-sectional study by Babio *et al.* investigating adherence to a traditional Mediterranean diet (using a 14-point score) and risk of metabolic syndrome, which is a cluster of common CVD risk factors, including central obesity, hyperglycaemia, low HDL-cholesterol levels, hypertension and hypertriglyceridaemia[41]. The traditional Mediterranean diet investigated by Babio *et al.* is characterised by a food pattern high in fruit, vegetables, grains and unsaturated fat and low in saturated fats[42]. This food pattern is comparable with the five food and nutrient indicators of the DQI used in this present study to investigate adherence to the Danish FBDG. Babio *et al.* found that a higher adherence to a traditional Mediterranean diet was associated with lower odds of having metabolic syndrome. In addition, Babio *et al.* found that subjects in the fourth quartile of the Mediterranean diet adherence, when compared with subjects in the lowest quartile of adherence to the Mediterranean diet, had 47 and 54 % lower odds of having low HDL-cholesterol levels and high TAG levels, respectively. However, in contrast to the low-risk middle-aged study participants of the present study, Babio *et al.* included 808 elderly high cardiovascular risk participants of the Reus PREDIMED Centre.

BP is known to be a variable measurement, and it can be hard to measure accurately. The surprising and unexpected finding of a positive association between the DQI and systolic BP is not in line with previous studies. In a meta-analysis of seventeen randomised controlled trials investigating the effect of dietary patterns on BP in adults, it was found that healthy dietary patterns such as the Nordic diet, the Mediterranean diet and the Dietary Approaches to Stop Hypertension decreased systolic and diastolic BP[43].

When comparing the results of the previously mentioned studies and the results of the present study, it is important to be aware of differences between first and foremost the study populations, as some were middle-aged with low CVD risk[39], and some older with features of the metabolic syndrome and therefore were at high CVD risk[41], all of which can have an effect on the results on cardiometabolic risk factors. Moreover, awareness of the different methods of dietary assessment is important, as it could play a role for the accuracy of estimating dietary intake[19,38,39,41]. Furthermore, the DQI used in the present study include various factors of more or less importance for cardiometabolic risk factors. In particular, the score of whole-grain intake included in the DQI is of importance, as a high whole-grain intake is associated with lower risk of CVD[44]. In addition, looking at the individual scores of the five food and nutrients included in the DQI, the median score for added sugar was higher than the other median individual scores for whole grains, fish, fruit and vegetables and saturated fat, indicating a higher compliance to this specific dietary guideline of a reduced sugar intake.

Using a dietary index approach enabled us to describe associations between food and nutrient intake in individuals with dietary patterns more or less compliant with the current Danish FBDG and cardiometabolic risk factors. Overall, the present cross-sectional study adds to the growing body of evidence that adherence to different national FBDG and diets rich in fruit, vegetables, whole grains, legumes and fish and low in meat and sweets are associated with a better cardiometabolic risk profile.

In conclusion, higher adherence to the current Danish FBDG, assessed by a DQI comprising of five components, was associated with a more beneficial cardiometabolic risk profile in a Danish adult population with a minimum of one self-rated risk factor for IHD. The DQI was inversely associated with BMI, trunk fat, hsCRP and glycaemic biomarkers in men.

The results substantiate the use of the DQI to measure adherence to the current Danish FBDG and associations with cardiometabolic risk factors, and indicate that adherence to the Danish FBDG may be beneficial for prevention of CVD. The results of this study further highlight the need to conduct sex-stratified analyses on CVD risk in this particular target group.

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The author contributions are as follows: J. L. A., K. O. and I. T. formulated the research question; J. L. A., C. H. and I. T. contributed to the study design; J. L. A. performed the statistical analyses; J. L. A., C. H., E. W. A., K. O. and I. T. were all involved in the interpretation of the data and critical revision of the manuscript; J. L. A. drafted the manuscript and all authors approved the final version.

None of the authors has any conflicts of interest to declare.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114517003695

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Paper II:

Short and long term dietary effects of applying Substitution dietary guidelines and Official dietary guidelines in a Danish adult population: The DIPI randomised controlled trial

Short and long term dietary effects of applying Substitution dietary guidelines and Official dietary guidelines in a Danish adult population: The DIPI randomised controlled trial

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Short title: Dietary effects of dietary guidelines

Keywords: Dietary record, ischemic heart disease, food-based dietary guidelines, dietary intake, dietary composition

Abstract

Addressing modifiable risk-factors such as diet can help prevent ischemic heart disease. The objective was to examine short- and long-term dietary effects of applying Substitution Dietary Guidelines (DG) and Official DG in a Danish adult population. A 6-months randomised, single-blinded parallel intervention study was conducted with 6 months follow-up. At baseline 219 participants were randomly assigned to either I) Substitution DG, II) Official DG, or III) habitual diet. At baseline and at 6 and 12 months dietary records, plasma alkylresorcinols concentrations, anthropometrics, and background questionnaires were collected. Linear regression analyses were applied. Compared to the participants on habitual diet, the Substitution DG group increased their intake of whole grain (17g/10MJ/d, 95%CI:6, 28 g/10MJ/d), dietary fibre (0.26g/MJ/d, 95%CI:0.09, 0.44g/MJ/d), fine vegetables (41g/10MJ/d, 95%CI:5, 7g/10MJ/d), and decreased their E% SFA (-1.51E%, 95%CI:-2.31,-0.70E%) and the Official DG group decreased E% SFA (-0.89E%, 95%CI: -1.69,-0.09E%). After 12 months, when compared to the habitual diet, both the Substitution DG group and the Official DG group increased intake of WG (16g/10MJ/d, 95%CI: 6, 27g/10MJ/d and 13 g/10MJ/d, 95%CI: 3, 23g/10MJ/d, respectively) and fish (23g/10MJ/d, 95%CI: 3, 43g/10MJ/d and 24g/10MJ/d, 95%CI: 5, 44g/10MJ/d, respectively), the Official DG group decreased E% SFA (0.84E%, 95%CI:-1.69,-0.001E%). In conclusion from baseline to 6 months, when compared to the habitual diet, the Substitution DG was more effective than the Official DG in changing the number of dietary components, resulting in a dietary composition of the overall diet being more cardio-protective. However, from baseline to 12 months the two DGs showed similar effectiveness.

Introduction

Ischemic heart disease (IHD) is a leading cause of morbidity and mortality worldwide with an estimated 7.4 million deaths due to IHD in 2015 [1]. Addressing modifiable risk factors such as an unhealthy diet can help in the prevention of IHD [2–4].

In the Nordic and European countries science based health messages including Dietary Reference Values and Dietary Guidelines (DG) are much alike [5]. The guidelines, including the Danish Official DG are based on systematic literature reviews of the associations between food intake and relevant nutrition-related diseases in a general population. These DGs are thus targeted most public health relevant nutrition-related diseases. However, DG may also be developed to target only one specific nutrition-related disease, e.g. IHD.

DG may be communicated in general terms, such as ´eat more or eat less´, with an additional quantification of the food and nutrients included in the DG [6]. Moreover, both cohort studies and randomised controlled studies have shown a lower risk of IHD when saturated fat (SFA) is substituted with polyunsaturated fat (PUFA)[7, 8]. Also, it has been suggested that replacement of SFA with carbohydrates with low glycaemic index (GI) values may be associated with a lower risk of IHD, whereas replacing SFA with carbohydrates with high GI values may be associated with a higher risk of IHD [9]. Furthermore, it is known that when individuals change their dietary intake of specific foods, they primarily change their dietary composition rather than their total energy intake [10]. Therefore, the substitution aspect is important and emphasise the importance of in dietary guidelines also to explicitly specifying dietary substitutions.

Since intake of food and nutrients vary between the dietary assessments methods, the validity of estimating the dietary intake may differ. The method of use for any study should be based on the food or nutrients of interest and the capacity of the target group to provide the needed details. The Danish diet is characterised by a relatively high intake of whole grain (WG) [11]. Furthermore, consumption of WG as a part of a healthy diet has consistently been shown to be associated with a lower risk of developing several diseases including cardiovascular diseases (CVD) [12, 13]. Alkylresorcinols (ARs) are phenolic lipids, that have been suggested as objective biomarkers for WG wheat and rye intake [14, 15]. In addition, validation studies in adults have shown that plasma AR concentrations increase proportionally with AR and WG intake [16, 17] and therefore, AR have been used as biomarkers in endpoint studies [18, 19]. Due to high relevance of

WGs in human health, it is relevant to validate estimated WG intake against an objective biomarker such as AR.

The primary objective of this study was to examine the short- and long term dietary effects of applying Substitution DG and Official DG in a Danish adult population at risk of ischemic heart disease. A secondary objective was to validate the dietary assessment method for WG intake using an objective biomarker.

Method

Study design

A 6-month randomised, single-blinded parallel intervention study was conducted from March 2014 to May 2015 in a real-life setting with adult participants with a minimum of 1 risk marker of IHD, with a 6-month follow-up. At baseline participants were assigned to 1 of 3 study groups I) food-based Substitution DG II) food-based Danish Official DG, or III) a habitual diet (**Figure 1**).

Short- and long term dietary effects of the guidelines were defined as the changes in diet from baseline to 6 months (end of the intervention) and from baseline to 12 months (follow-up), respectively.

The study was a part of the research project ´Diet and Prevention of Ischemic Heart Disease – a Translational Approach´ (DIPI) (www.DIPI.dk).

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by The Capital Region of Denmark Ethics Committee (Journal no. H-1-2013-110) and by the Danish Data Protection Agency (Journal no. 2013-54-0571). Written informed consent was obtained from all study participants and they received a small remuneration of around 34 British pound (GBP) for their participation in the study. The study was registered at ClinicalTrials.gov (registry name “Diet and Prevention of Ischemic Heart Disease: a Translational Approach (DIPI)”, ID no. NCT02062424).

Study participants

Potential participants were identified using a unique personal identification number assigned to all Danish citizens in the Civil Registration System [20]. In total 5000 men and women born 1949-1984 and living in a defined area of greater Copenhagen were invited by letter to participate in the study. The number of invited participants was based on previous experience of a low response rate when

recruiting participants for long term interventions. Overall, 334 responded to the invitation and were screened using a self-administered questionnaire including questions on the inclusion- and exclusion criteria. The potential participants were asked to measure and report their height in meters, weight in kilo grams (kg), their waist circumference 2 cm above their umbilicus, and whether or not they were physically active for more than 15 minutes per week. Furthermore, the self-administered questionnaire included questions on the exclusion criteria. The eligible participants were invited to an information meeting, which included an introduction to the web-based dietary assessment software.

The inclusion criteria were age between 30 and 65 years, and a minimum of 1 self-rated risk factor for IHD, i.e. overweight or obesity (BMI ≥ 25), waist circumference ≥80 cm for women and ≥94 cm for men, and/or physical inactivity defined as being moderately physically active in leisure time for 15 minutes or less per week.

The exclusion criteria were current smoking, pregnancy or plans to become pregnant within the next 12 month, breastfeeding, history of CVD, type-2 diabetes, chronic disease/disorders that could affect the results of the study (the chronic diseases that the subjects reported were evaluated by the physician in charge), drug abuse within the last 12 months, regular alcohol consumption >21 units/week for men or >14 units/week for women, allergies or intolerance of the food groups included in the DG, consumption of dietary supplements with high doses of nutrients that could have a potential effect on IHD risk factors (e.g. fish oils) and/or no access to a computer and internet.

Randomisation and intervention

A schematic overview of the study design is presented in **Figure 1**. After the baseline examination the participants were randomly assigned to one of the 3 study groups using a computer randomisation plan (www.randomization.com) for men and women separately to ensure that the randomisation was balanced by sex.

The guidelines given to the participants in the Substitution DG group focused on 5 of the 10 Danish Official DG (**Table 1**) where the scientific evidence for a relationship between a dietary factor and an IHD outcome was found to be convincing or probable [(21)].

The guidelines given to the participants in the Official DG group included 10 guidelines on food, beverages, and physical activity (**Table 1**). The Official DG were updated based on a systematic

literature review and on knowledge regarding Danish food habit and relevant nutrition-related diseases [21].

The two study groups assigned to receive either the Substitution DG or the Official DG, were given information about the DG via a letter including a leaflet with the respective guidelines and via a homepage (www.dipi.food.dtu.dk). The participants were given a personal password to the homepage where they could find leaflets and recipes. The leaflets and recipes for the Substitution DG and the Official DG were identical in design, structure and numbers, and therefore it was only the content that varied depending on which DG the participant was randomised to receive information about. All recipes given to the intervention groups were developed by the Danish Veterinary and Food Administration and Keyhole nutrition labelled [22] (a nutrition label developed to make it easier for Nordic consumers to select healthy products). To increase motivation and compliance to the respective DG, an email with two new recipes for each of the two study groups was send out to the participants biweekly during the intervention period. In the group receiving the Substitution DG, only recipes with fish dishes were sent out. To increase motivation and compliance in the habitual diet group the participants were also sent an email every second week, including a 'thank you for still participating' greeting.

At the end of the intervention, after 6 months the participants were told that the intervention study was finished, but that they would be re-invited to a follow-up examination after further 6 months (**Figure 1**).

Dietary assessment

The study participants recorded their dietary intake using a web-based dietary assessment software for seven consecutive days [23]. A user manual for the software was given to the participants at baseline. The web-based dietary assessment software was originally developed and validated for children aged 8-11-years and slightly customised to fit the adult study population of the DIPI study [23–25]. At least four days of food reporting had to be completed for inclusion of the study participants in the analysis [26].

The dietary assessment software was structured according to a typical Danish meal pattern covering breakfast, lunch, dinner and three in-between meals. The participants could estimate the amount consumed by selecting the closest portion size among four different digital images in 80 photograph series. Reminders for frequently forgotten foods (e.g. spreads, sugar, sauces, dressings, snacks, candy and beverages) were included. Furthermore, the participants reported the intake of nutritional

supplements and whether a day represented a usual or an unusual intake, including reasons for unusual intakes such as illness. If a participant failed to report for a day, the participant was reminded by an e-mail the next day [23].

Intakes of food items, energy and nutrients were calculated for each study participant as an average of seven days using the software system General Intake Estimation System (GIES) version 1.000.i6 (National Food Institute, Technical University of Denmark, Kgs. Lyngby, DK) and the Danish Food Composition Databank version 7.0 (National Food Institute Technical University of Denmark, 2009).

Under- and over-reporters

Under- and over-reported energy intake was defined according to a ratio of reported mean energy intake to basal metabolic rate (EI;BMR) and classified by cut-offs suggested by Black [27, 28]. Under-reporters were defined as EI:BMR $\leq$ 1.05 and over-reporters was defined as EI:BMR $\geq$ 2.28, using a physical activity level 1.55 (data not shown).

Anthropometric measurements (height, weight, and waist circumference)

Height was measured to the nearest 0.5 cm on a wall mounted Stadiometer (SECA, Hamburg, Germany). Fasting body weight in kg and trunk fat was registered on a fat analysis weight (Tanita BC 418 MA, Tokyo, Japan). Waist- and hip circumference were measured twice, with an anthropometric tape (SECA 201, Hamburg, Germany) and the average was reported.

Body mass index (BMI) was defined as weight in kg divided by squared height in meters (kg/m^2).

Background questionnaires

Lifestyle questionnaires were used to obtain information about the participant's education level (primary school/high school, associate degree, under-graduate, graduate) and the level of physical activity at leisure time (extremely active, moderately active, sedentary or inactive). The question about the level of physical activity was based on one question about the study participants physical activity during leisure time the past 6 month and was based upon the Danish National Health Profile questionnaire [29].

Plasma alkylresorcinol concentrations

Alkylresorcinol homologs (C17:0, C19:0, C21:0, C23:0, C25:0, and their sum) were extracted and purified from plasma samples and analysed by a gas chromatography–mass spectrometry (Finnigan

TM Trace GC Ultra Gas chromatograph coupled to a Finnigan Trace DSQ II mass detector, Thermo Fisher Scientific, Waltham, MA, USA)according to the method is described in detail elsewhere [30].

Statistical method

Statistical power calculations based on evidence from previous similar studies [31–33] were used to estimate that 62 subjects in each intervention arm was sufficient to detect a difference of 0.25 mmol/L low-density lipoprotein (LDL) cholesterol (SD, 0.49) ($\alpha = 0.05$, $\beta = 0.8$). In order to allow for a drop-out of 20 percent the number of participants was set to a total of 225. Baseline characteristics and dietary intake of the study participants were summarised for each intervention group using medians and 50 % central range for continuous variables and proportions for categorical variables.

Changes in dietary composition, energy intake and energy distribution in the Substitution DG group and the Official DG group, compared to the habitual diet from baseline to 6 months and from baseline to 12 months were based on two multiple liner regression models. Model 1 was adjusted for baseline intake of the outcome variable, and model 2 further adjusted for sex, age group (<50 and ≥50) and BMI group (18.5-25 = Normal weight, >25-30 = Overweight, >30 = Obese). In Model 2 we additionally tested for statistically significant interactions between intervention group and sex, intervention group and age group, and intervention group and BMI group. These interactions were tested to investigate whether the intervention had different effects for men and women, for participants above or below the 50 years of age or for normal weight, overweight or obese study participants. If an interaction was statistically significant, separate results according to the level of the effect modifier were provided. Furthermore, sensitivity analysis excluding under- and over-reporters were made to investigate the impact of under- and over-reported energy intake on the changes in dietary composition and energy distribution in the two DG groups, when compared to the habitual diet.

To check the model assumptions the standardised residuals of the final models were examined for normality, variance homogeneity and linearity.

WG intake and total AR concentration in plasma were grouped into quartiles and cross-tabulation for total WG intake, and AR concentrations in plasma were presented to examine the agreement between quartiles using the baseline data. To validate the WG intake estimated form the web-based dietary assessment software a simple linear regression model was used to test the association between WG intake and total AR concentrations in plasma at baseline. To normalise the distribution

of the residuals and to improve variance homogeneity, AR concentrations and WG intake were logarithm2 transformed.

The statistical analyses were carried out using RStudio (Version 0.99.441 – © 2009-2015 RStudio, Inc.). Statistical significance was established at $p < 0.05$.

Results

Baseline characteristics of the participants

A total of 222 participants met the inclusion and exclusion criteria and were enrolled into the study, which was 67% of the initially screened potential study participants. Three participants dropped out of the study before randomisation (a full baseline examination was missing) and they were therefore excluded, see flow chart (**Figure 2**). Baseline characteristics of the remaining 219 study participants, by randomised intervention group are presented in **Table 2**. Altogether, 199 completed the intervention, corresponding to a compliance of 90 % and 186 participants completed the follow-up. Reasons for non-compliance are given in the flow chart in **Figure 2**.

Diet-differences from baseline to 6 months

Table 3 shows the composition of the participant's usual diets at baseline (median (p10- p90)) with all three groups combined and the between group differences in changes in diet from baseline to 6 months.

Substitution DG compared to the habitual diet

Compared to the habitual diet, the group receiving the Substitution DG increased their intake of WG (17 g/10 MJ/d, 95%CI: 6, 28 g/10MJ/d), dietary fibre (men; 0.31 g/MJ/d , 95%CI: 0.04, 0.58 g/MJ/d, and women 0.23 g/MJ/d, 95%CI: 0.002, 0.45 g/MJ/d), fine vegetables (41 g/10MJ/d, 95%CI: 5, 77 g/10MJ/d), and decreased their percentage of energy (E%) intake from SFA (-1.51 E%, 95%CI:-2.31, -0.70 E%). In addition, women in the group increased their overall intake of vegetables (69.98 g/10MJ/d, 95%CI: 19.37, 120.58 g/10MJ/d) and normal weight study participants in the group increased their E% intake from PUFA (1.55 E%, 95%CI: 0.73, 2.36 E%).

Official DG compared to the habitual diet

Compared to the habitual diet, the group receiving the Official DG decreased their E% intake from SFA (-0.89 E%, 95%CI: -1.69, -0.09 E%). Furthermore, the women in the group increased their overall intake of vegetables (60.31 g/10MJ/d, 95%CI: 10.16, 110.45 g/10MJ/). Moreover, men in the group decreased their intake of coarse vegetables (-40 g/10MJ/d, 95%CI: -78, -2 g/10MJ/d).

Diet-differences from baseline to 12 months

Table 4 shows the between group differences in changes in diet from baseline to 12 months.

Substitution DG compared to the habitual diet

Compared to the habitual diet, the group receiving the Substitution DG continued to have an increased intake of WGs from baseline to 12 months (16 g/10MJ/d, 95%CI: 6, 27g/10MJ/d) and increased their intake of fish (23 g/10MJ/d, 95%CI: 3, 43 g/10MJ/d). For vegetables intake in general, obese study participants in the group increased their intake (166 g/10MJ/d, 95%CI: 45, 287 g/10MJ/d). Moreover, the group increased their intake of sugar and candy (9 g/10MJ/d, 95%CI: 0.2, 17g/10MJ/d) and participants 50 years or above increased their E% intake from carbohydrate (2.67 E%, 95%CI: 0.15, 5.19 E%).

Official DG compared to the habitual diet

Compared to the habitual diet, the group receiving the Official DG had from baseline to 12 months increased their intake of WG (13 g/10MJ/d, 95%CI: 3, 23 g/10MJ/d) and fish (24 g/10MJ/d, 95%CI: 5, 44 g/10MJ/d) and they continued to have a decreased E% intake from SFA (-0.84 E%, 95%CI: -1.69, -0.001 E%). Furthermore, participants 50 years or above in the group increased their E% intake from carbohydrates (2.95 E%, 95%CI: 0.45, 5.46 E%).

Under- and over-reporters

At baseline, at 6 months and at 12 months 21%, 35% and 31%, respectively of the participants were classified as under-reporters and 1%, respectively as over-reporters. Of the under-reporters 86-90% were overweight or obese, 41-44% of the under-reporters were men and all of the over-reporters were men.

Excluding under- and over-reporters for the sensitivity analysis did not change the conclusion for the two sets of DG, when compared to the habitual diet.

Association between whole grain intake and plasma AR at baseline

From the cross-classification between reported WG intake and AR concentrations, 36% of the study participants were classified in the same quartile, 70% were classified in the same quartile or adjacent quartile, 22% were 2 quartiles apart, and 9% were misclassified in the opposite quartile (data not shown). A statistically significant association between WG intake and plasma AR was observed at baseline ($p < 0.0001$).

Discussion

In the present study we found that from baseline to 6 months, when compared to the habitual diet, the Substitution DG was more effective than the Danish Official DG in changing the number of dietary components, resulting in a dietary composition of the overall diet being more cardio-protective. However, from baseline to 12 months the effectiveness in changing the dietary composition towards a more cardio-protective diet was similar for both the Substitution DG and the Official DG in form of how many dietary components have changed, when compared to the habitual diet.

Overall, from baseline to 6 months, the group assigned to follow the Substitution DG increased their intake of WG, dietary fibre and fine vegetables and decreased their E% intake from SFA, when compared to the habitual diet. Furthermore, women increased their overall vegetable intake and normal weight study participants increased their E% intake from PUFA, when compared to the habitual diet. The group assigned to follow the Official DG decreased their E% intake from SFA, when compared to the habitual diet. Further, women increased their intake of vegetables in general, like the women in the Substitution DG group, and men decreased their intake of coarse vegetables.

From baseline to 12 months, the group receiving the Substitution DG continued to have an increased intake of WG. The group assigned to follow the Official DG had now, in contrast to from baseline to 6 months, also increased their intake of WG, when compared to the habitual diet. Changes in dietary fibre intake did not stay significant from baseline to 12 months in the Substitution DG group and besides an increased general intake of vegetables of obese study participants in the Substitution DG group, no other changes in total-, fine-, or coarse vegetables was found from baseline to 12 months in either of the two DG groups, when compared to the habitual diet.

When compared to the habitual diet, the participants in both the two DG groups had from baseline to 12 months, in contrast to from baseline to 6 months, increased their intake of fish. One of the Substitution DG included the specific wording ´eat fish instead of red meat´, however, only a tendency towards a change in red meat intake was found in the Substitution DG group, when compared to the habitual diet. The fact that we did not observe an increase in fish intake in either of the two DG groups from baseline to 6 months, even though both DG groups include advice to increase fish intake, could be explained by the fact that it may be difficult for people to exchange red meat with fish in many dishes. In many of the dishes including red meat, it would make little

sense to simply replace the meat with fish, and therefore participants are forced to change the whole dish instead of just one food component. It could be speculated that it will take some time to integrate new food/meal habits explaining why we only see an increase in fish intake from baseline to 12 months.

As individuals usually change the dietary composition to maintain energy balance [10], the higher intake of the food groups found in the present study may be expected to replace other energy-providing foods in the diet, which may have beneficial, neutral or detrimental effects. In the present study we did not find any decrease in certain food groups, even though we did not find any significant difference in total energy intake in either of the two DG groups, when compared to the habitual diet. However, we found that both the DG groups decreased their E% intake from SFA (corresponding to a 12 % and 7 % decreased intake from SFA from baseline, respectively) from baseline to 6 months. In addition, from baseline to 12 months the group assigned to follow the Substitution DG had a tendency towards a decreased E% intake from SFA and the group assigned to follow the Official DG had a decreased E% intake from SFA (corresponding to a 5 % and 6 % reduced intake from SFA from baseline, respectively). The decrease in SFA could be explained by the statistical tendency of a decreased meat intake in both the two DG groups and the tendency of a decreased cheese intake in the group assigned to the Substitution DG. Also, participants in the Substitution DG group with normal weight had increased their E% intake from PUFA from baseline to 6 months (corresponding to a 12 % increased intake form PUFA from baseline), when compared to the habitual diet; however from baseline to 12 months the increase in E% intake from PUFA was not significant anymore.

Replacing dietary SFA with PUFA and WG have been shown in both prospective studies and randomized controlled trials to be beneficial for cardiovascular health [5, 8, 9, 34, 35]. Mozaffarian et al. found in a meta-analysis of 8 RCT that when 5 E% from SFA was replaced by an equal amount of PUFA, LDL cholesterol and total cholesterol to high-density lipoprotein (HDL) cholesterol-ratio was reduced [8]. This finding is supported by another systematic review including regression analysis of 84 RCT by Mensink et al. who found that when 1 E% from SFA was replaced with an equal amount of PUFA, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, LDL cholesterol to HDL cholesterol-ratio and triglyceride to HDL cholesterol ratio were reduced [34]. Moreover, Mensink et al. showed that when 1 E% from SFA was replaced with carbohydrates, a decrease in total cholesterol, LDL cholesterol, HDL cholesterol and LDL cholesterol to HDL

353 cholesterol-ratio was seen, but they also found an increase in triglycerides. However, the source of
354 carbohydrates is suggested to be important, as replacing SFA with refined starches/added sugars
355 have shown not to be protective against CVD, whereas replacing SFA with WG have shown to be
356 protective against CVD [5, 35].

357 Li et al. suggest that when decreasing SFA intake, most people appear to increase the intake of low-
358 quality carbohydrates, such as refined starches and/or added sugar, rather than increase the intake of
359 unsaturated fats [35]. This suggestion by Li et al. is in line with findings of the present study where
360 we found a decreased E% intake from SFA or a tendency towards a decreased E% intake from SFA
361 in both DG groups from baseline to 6- and 12 months, when compared to the habitual diet. In
362 addition, in the present study we also found that participants 50 years or above in both DG groups
363 increased their E% from carbohydrates from baseline to 12 months, when compared to the habitual
364 diet. Also, the group receiving the Substitution DG had from baseline to 12 months, in contrast to
365 from baseline to 6 months, increased their intake of sugar and candy. A meta-analysis of 40 RCT
366 found that higher intake of sugar significantly increased TAG, total-cholesterol, LDL-cholesterol
367 and HDL-cholesterol [36].

368 The substitution aspect of DGs is important because it approaches a real-life setting by specifying
369 what to eat instead of a certain food or nutrient item. In addition, because the Substitution DG only
370 consisted of 5 food and nutrient guidelines, they could be expected to be easier to follow and
371 implement in the everyday life of the participants than the Official DG consisting of ten guidelines.
372 However, the long term dietary effect from baseline to 12 months of both the Substitution DG and
373 the Official DG were similar in form of changes in the numbers of dietary components, when
374 compared to the habitual diet

375 Misreporting as under- and over-reported energy intake in self-reported dietary assessment methods
376 could be a potential source of bias [37]. However, in the present study excluding under- and over-
377 reporters for the sensitivity analysis did not change the conclusion for the two sets of DG, when
378 compared to the habitual diet.

379 In the present study, self-rated WG intake was associated with plasma AR at baseline. Andersen et
380 al. have suggested that plasma AR concentrations in fasting samples can be used as a biomarker of
381 WG intake in free-living populations with a high and consistent WG intake, comparable with the
382 study population in the present study [17]. Furthermore, from cross-classification between reported

WG intake and AR concentrations we found that 70% were classified in the same quartile or adjacent quartile, which is in agreement with findings from two other studies by Ross et al. [14] and Biltoft et al. [23].

Our study has some limitations. To some extent the present study does not present the direct/actual effects of the DGs on dietary composition, as ensuring adherence to DGs is difficult in long-term intervention trial, both because of prohibitive costs and of what you impose on the study participants in a long-term trial. However, the present study does provide evidence for the dietary effects of advising two different sets of DGs; one focusing on specific substitutions targeted primary prevention of IHD and one set of DGs with a wording more traditional in the European and Nordic countries, namely the Danish Official DG, which focuses on nutrition-related diseases relevant in Denmark. A strength of this study is the real-life setting, where participants were free-living and empowered to modify their dietary pattern. Also, the study was conducted in a healthy adult population, including men and women, non-smoking, not taking blood pressure- or hyperlipidemia medication, which is a strength because evidence is lacking for primary prevention of CVD. In addition, although some of the found diet and nutritional changes are small, they are almost all in the direction of improvement. Moreover, the found improvements in dietary effects are the result of simply providing people with DG over a 12 month period. Last but not least, validation of the dietary method used and evidence for compliance to the intervention were provided through an objective biomarker of intake.

In conclusion from baseline to 6 months, when compared to the habitual diet, the Substitution DG was more effective than the Danish Official DG in changing the number of dietary components, resulting in a dietary composition of the overall diet being more cardio-protective. However, from baseline to 12 months the effectiveness in changing the dietary composition towards a more cardio-protective diet was similar for both the Substitution DG and the Official DG in form of changes in numbers of dietary components, when compared to the habitual diet. Furthermore, self-rated WG intake was associated with plasma AR at baseline, objectively validating WG-intake

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Conflict of Interest

None.

Authorship

The authors contributions are as follows: JLA, KO and IT formulated the research question; JLA, CH and IT contributed to the study design; JLA performed the statistical analyses, JLA, CH, RA, EWA, RL, KO and IT were all involved in the interpretation of the data and critical revision of the manuscript; JLA drafted the manuscript and all authors approved the final version.

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Table 1: The Substitution dietary guidelines and the Danish Official dietary guidelines

Substitution dietary guidelines	Official dietary guidelines
	Eat a variety of foods, but not too much, and be physically active
Eat fruit instead of candy and cake	Eat fruits and many vegetables
Eat coarse vegetables instead of fine vegetables†	
Eat fish instead of red meet	Eat more fish
Eat wholegrain products instead of products with no wholegrain grains	Choose whole grains
	Choose lean meats and cold meats
	Choose low-fat dairy products
Eat unsaturated fat instead of saturated fat	Eat less saturated fat
	Eat foods with less salt
	Eat less sugar
	Drink water

† Vegetables are classified from type of food groups (e.g. all types of cabbage, rooted vegetables and onions are classified as coarse vegetables and all vegetables with a high water content like tomatoes and salad are classified as fine vegetables)

Table 2: Baseline characteristics of the study participants by randomised intervention group (n=219).

Participants characteristics	Habitual diet (n=73)		Substitution DG (n=74)		Official DG (n=72)	
	Median	(p25-p75)	Median	(p25-p75)	Median	(p25-p75)
Age (years)	51.0	(42.0, 55.0)	51	(42.3, 57.0)	52.5	(45.0, 58.0)
Women, % (n)	59	(43)	58	(43)	60	(43)
Weight (kg)	85.2	(71.8, 90.8)	82.2	(74.2, 88.8)	80.7	(70.5, 91.8)
BMI†	26.0	(24.2, 29.3)	27.0	(25.6, 29.2)	26.8	(24.6, 29.4)
Weight status†:						
Normal weight, % (n)	33	(24)	22	(16)	28	(20)
Overweight, % (n)	51	(37)	56	(42)	51	(37)
Obese, % (n)	16	(12)	22	(16)	21	(15)
Waist circumference (cm)	92.3	(85.7, 99.4)	92.3	(86.8, 98.2)	94.1	(83.1, 99.2)
Hip circumference (cm)	106.9	(103.7, 112.4)	107.2	(104.5, 114.5)	107.1	(102.8, 112.7)
Educational level						
Primary school or high school, % (n)	22	(16)	26	(19)	29	(21)
Associate degree, % (n)	11	(8)	8	(6)	6	(4)
Undergraduate school, % (n)	37	(27)	42	(31)	40	(29)
Graduate school, % (n)	30	(22)	24	(18)	25	(18)

DG, dietary guidelines; BMI, Body Mass Index.

†BMI is calculated as weight in kilograms divided by the square of height in meters (kg/m2). 18.5-25 = Normal weight, 25-30 = Overweight, >30 = Obese.

570 Table 3: Between group differences in changes in energy adjusted diet composition (g/10MJ/day), total energy (MJ/day), energy
571 contribution (E%) of macronutrients, dietary fibre (g/MJ) from baseline to after 6 months.

			Model 1†				Model 2‡			
	Baseline intake (n=219) Median (p10-p90)		SUB DG vs. habitual Mean between group difference, 95%CI		OFF DG vs. habitual Mean between group difference, 95%CI		SUB DG vs. habitual Mean between group difference, 95%CI		OFF DG vs. habitual Mean between group difference, 95%CI	
Diet composition (g/10MJ/day):										
Bread and cereals	218	(141-301)	6	(-15, 26)	-3	(-23, 18)	6	(-14, 26)	-2	(-22, 18)
Whole grains	61	(31-111)	17**	(6, 28)	6	(-4, 17)	18**	(7, 28)	7	(-4, 17)
Meat and meat products	139	(59-249)	-15	(-38, 8)	-15	(-38, 8)	-16	(-39, 6)	-15	(-38, 7)
Poultry and poultry products	32	(0-102)	10	(-7, 28)	4	(-13, 20)	-4	(-36, 27)	-10	(-39, 20)
Fish and fish products	46	(5-111)	12	(-6, 30)	15	(-4, 33)	12	(-7, 30)	15	(-4, 33)
Fruit and fruit products	143	(43-331)	25	(-13, 63)	22	(-16, 60)	26	(-12, 64)	23	(-15, 62)
Vegetables and vegetables products	220	(110-425)	44*	(5, 83)	16	(-23, 55)	45*	(5, 84)	16	(-23, 55)
Women							70**	(19, 121)	60*	(10, 110)
Men							8	(-52, 69)	-48	(-109, 12)
Vegetables, coarse§	97	(29-208)	8	(-17 to 33)	-4	(-28, 21)	9	(-15, 334)	-3	(-27, 22)
Women							18	(-14, 50)	23	(-9, 54)
Men							-4	(-42, 34)	-40*	(-78, -2)
Vegetables, fine§	114	(49-228)	28*	(1, 56)	15	(-12, 42)	41*	(5, 77)	34	(-2, 70)
Potatoes and potatoes products	57	(7-138)	-9	(-30, 11)	6	(-14 , 26)	-9	(-30, 11)	5	(-15, 25)
Milk and milk products	243	(68-513)	-4	(-63, 56)	38	(-21, 96)	-6	(-65, 53)	38	(-21, 96)
Cheese and cheese products	45	(11-129)	-17	(-36, 2)	-16	(-35, 2)	-18	(-37, 1)	-18	(-36, 1)
Edible fats			-4	(-8, 1)	-3	(-7, 2)	-4	(-8, 1)	-3	(-7, 2)
Sugar and candy	35	(10-77)	4	(-4, 12)	2	(-6, 11)	5	(-3, 13)	3	(-5, 11)
Total energy, energy contribution of macronutrient, dietary fibre										
Energy, MJ	8.6	(5.8-12.4)	-0.12	(-0.76, 0.51)	0.06	(-0.58, 0.70)	-0.84	(-3.73, 2.05)	1.53	(-1.28, 4.34)
Energy from protein, %	17	(14-22)	-0.40	(-1.25, 0.46)	-0.12	(-0.97, 0.73)	-0.50	(-1.34, 0.34)	-0.17	(-1.01, 0.66)
Energy from carbohydrate, %	44	(35-53)	1.60	(-0.43, 3.63)	1.66	(-0.35, 3.68)	1.77	(-0.23, 3.78)	1.83	(-0.16, 3.81)
Energy from added sugar, %	7	(2-13)	-0.08	(-1.18, 1.03)	0.03	(-1.06, 1.13)	0.02	(-1.08, 1.12)	0.09	(-1.01, 1.19)
Energy from total fat, %	35	(29-42)	-1.35	(-3.04, 0.34)	-1.01	(-2.68, 0.67)	-1.35	(-3.05, 0.36)	-1.03	(-2.72, 0.66)
Energy from SFA, %	13	(11-17)	-1.43***	(-2.24, -0.63)	-0.86*	(-1.65, -0.06)	-1.51***	(-2.3, -0.70)	-0.89*	(-1.69, -0.09)
Energy from MUFA, %	13	(10-17)	-0.28	(-1.19, 0.63)	-0.20	(-1.11, 0.71)	-0.22	(-1.13, 0.70)	-0.19	(-1.10, 0.72)

Energy from PUFA, %	5	(4-7)	0.43	(-0.004, 0.87)	0.02	(-0.42, 0.45)	0.47*	(0.04, 0.91)	0.03	(-0.40, 0.46)
Normal weight							1.55***	(0.73, 2.36)	0.05	(-0.71, 0.81)
Overweight							-0.06	(-0.64, 0.51)	-0.03	(-0.61, 0.55)
Obese							0.60	(-0.42, 1.63)	0.11	(-0.95, 1.18)
Energy from alcohol, %	5	(1-14)	0.52	(-0.66, 1.69)	-0.43	(-1.62, 0.76)	0.50	(-0.67, 1.67)	-0.48	(-1.67, 0.70)
Dietary fibre, g/MJ/day	2	(2-3)	0.24**	(0.07, 0.42)	0.06	(-0.11, 0.24)	0.26**	(0.09, 0.44)	0.08	(-0.09, 0.25)
Women							0.23*	(0.01, 0.45)	0.22	(-0.01, 0.44)
Men							0.31*	(0.04, 0.58)	-0.12	(-0.39, 0.15)

572 SUB DG, substitution dietary guidelines; habitual, habitual diet; OFF DG, Danish official dietary guidelines; g, gram; MJ, mega joule;
573 SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids
574 Levels of significance are marked as: * p<0.05, ** p=<0.01, *** p=0.001
575 † simple liner regression models adjusted for baseline intake of the outcome variable
576 ‡ multiple liner regression model adjusted for baseline intake of the outcome variable, sex, age group (<50 and ≥50), BMI group (18.5-25 =
577 Normal weight, >25-30 = Overweight, >30 = Obese) and interactions between intervention group and sex, intervention group and age
578 group, and intervention group and BMI group
579 §Vegetables are classified from type of food groups (e.g. all types of cabbage, rooted vegetables and onions are classified as coarse
580 vegetables and all vegetables with a high water content like tomatoes and salad are classified as fine vegetables).
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588 Table 4: Between group differences in changes in energy adjusted diet composition (g/10MJ/day), total energy (MJ/day), energy
589 contribution (E%) of macronutrients, dietary fibre (g/MJ) from baseline to after 12 months.

	Model 1 †				Model 2 ‡			
	SUB DG vs. habitual Mean between group difference, 95%CI		OFF DG vs. habitual Mean between group difference, 95%CI		SUB DG vs. habitual Mean between group difference, 95%CI		OFF DG vs. habitual Mean between group difference, 95%CI	
Diet composition (g/10MJ/day):								
Bread and cereals	9	(-12, 29)	6	(-15, 26)	10	(-10, 31)	7	(-14, 27)
Whole grains	15**	(5, 26)	12*	(2, 22)	16**	(6, 27)	13*	(3, 23)
Meat and meat products	-22	(-47, 2)	-24*	(-48, -0.2)	-22	(-47, 2)	-24	(-48, 0.3)
Poultry and poultry products	-1	(-20, 18)	10	(-9, 28)	2	(-17, 20)	15	(-3, 33)
Fish and fish products	25*	(5, 45)	25*	(6, 45)	23*	(3, 43)	24*	(5, 44)
Fruit and fruit products	-3	(-41, 35)	1	(-37, 38)	-4	(-42, 34)	-1	(-39, 36)
Vegetables and vegetables products	-20	(-69, 29)	-13	(-62, 37)	-20	(-69, 30)	-11	(-61, 38)
Normal weight					-92	(-183, 0.1)	-71	(-156, 15)
Overweight					-36	(-101, 29)	6	(-60, 72)
Obese					166**	(45, 287)	61	(-62, 185)
Vegetables, coarse§	-15	(-45, 16)	-8	(-39, 23)	-14	(-45, 17)	-8	(-38, 23)
Vegetables, fine§	-8	(-40, 23)	-10	(-41, 21)	-8	(-39, 24)	-9	(-41, 22)
Potatoes and potatoes products	-8	(-34, 19)	13	(-12, 38)	-7	(-33, 20)	14	(-11, 39)
Women					14	(-20, 48)	4	(-28, 36)
Men					-37	(-77, 3)	29	(-8, 66)
Milk and Milk products	22	(-29, 74)	-1	(-52, 50)	19	(-33, 71)	-4	(-54, 47)
Cheese and cheese products	-22	(-47, 3)	-4	(-29, 21)	-23	(-48, 2)	-4	(-29, 21)
Edible fats	0.4	(-4, 5)	-1	(-6, 3)	0.1	(-4, 5)	-2	(-6, 3)
Sugar and candy^^	9	(1, 18)	3	(-5, 12)	9*	(0.2, 17)	3	(-5, 12)
Total energy, energy contribution of macronutrients and dietary fibre								
Energy, MJ	0.07	(-0.52, 0.66)	0.29	(-0.30, 0.88)	0.13	(-0.45, 0.71)	0.31	(-0.27,.89)
Energy from protein, %	-0.41	(-1.42, 0.60)	0.42	(-0.58, 1.43)	-0.44	(-1.46 , 0.57)	0.42	(-0.59, 1.43)
Energy from carbohydrate, %	0.96	(-1.00, 2.93)	0.86	(-1.09, 2.81)	1.03	(-0.95, 3.02)	0.90	(-1.07, 2.87)
<50 years					-1.29	(-4.40, 1.83)	-2.18	(-5.28, 0.92)
≥50 years					2.67*	(0.15, 5.19)	2.95*	(0.45, 5.45)

Energy from added sugar, %	0.93	(-0.17, 2.02)	0.10	(-0.99, 1.20)	0.86	(-0.24, 1.96)	0.07	(-1.02, 1.17)
Energy from total fat, %	0.15	(-1.48, 1.78)	-0.55	(-2.17, 1.07)	0.05	(-1.60, 1.69)	-0.63	(-2.26, 1.00)
Energy from SAF, %	-0.68	(-1.53, 0.17)	-0.82	(-1.66, 0.03)	-0.73	(-1.59, 0.12)	-0.84*	(-1.69, -0.001)
Energy from MUFA, %	0.43	(-0.50, 1.36)	0.08	(-0.85, 1.00)	0.44	(-0.51, 1.38)	0.06	(-0.87, 1.00)
Energy from PUFA, %	0.20	(-0.22, 0.62)	0.25	(-0.17, 0.67)	0.19	(-0.24, 0.61)	0.24	(-0.18, 0.66)
Energy from alcohol, %	-1.23	(-2.44, -0.03)	-1.18	(-2.39, 0.04)	-1.15	(-2.37, 0.07)	-1.11	(-2.33, 0.11)
Fibre, g/MJ/day	0.06	(-0.12, 0.24)	0.09	(-0.09, 0.27)	0.08	(-0.10, 0.26)	0.10	(-0.08, 0.28)

590 SUB DG, substitution dietary guidelines; habitual, habitual diet; OFF DG, Danish official dietary guidelines; g, gram; MJ, mega joule;
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Paper III:

Effects of targeted substitution dietary guidelines on ischemic heart disease risk factors in an adult Danish population: The DIPI randomised controlled trial.

Effects of targeted substitution dietary guidelines on ischemic heart disease risk factors in an adult Danish population: The DIPI randomised controlled trial

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Running title: Dietary guidelines and ischemic heart disease risk

Abstract

Background/Objectives: Addressing modifiable risk-factors such as diet can help prevent ischemic heart disease (IHD). The objective was to examine effects of applying targeted Substitution dietary guidelines (DG) on IHD risk factors in an adult Danish population with a minimum of one self-reported risk factor for IHD.

Subjects/Methods: In all 219 participants were at baseline randomly assigned to either I) targeted Substitution DG, II) Official DG, or III) Habitual diet in a six-month, single-blinded parallel randomised controlled trial, with a six months follow-up in a real-life setting. At baseline, at six months and at 12 months, dietary records, fasting blood samples analysed for concentrations of triglycerides, total cholesterol, high density lipoprotein-cholesterol, high-sensitivity C-reactive protein, glucose, haemoglobin A_{1c}, insulin and alkylresorcinols, blood pressure and heart rate, anthropometrics, and background questionnaires were collected. Linear regression analyses were applied.

Results: Compared to the Habitual diet, no overall differences in change in cardiometabolic risk factors were found in either of the two DG groups. A statistically significant decrease in waist circumference (-4.41 cm, 95%CI: -7.93, -0.88cm, p= 0.0145) from baseline to 12 month were found in obese study participants receiving the Official DG, when compared to the Habitual diet.

Conclusion: In conclusion, when compared with the Habitual diet, neither the Substitution DG targeting prevention of IHD nor the Danish Official DG showed any overall beneficial effects on IHD risk factors in an adult Danish population with a minimum of one self-reported risk factor for IHD in a free-living dietary advice randomised controlled trial.

Introduction

Ischemic heart disease (IHD) is the leading cause of morbidity and mortality worldwide with an estimated 7.4 million deaths due to IHD in 2015 [1,2].

Addressing behavioural risk factors such as an unhealthy diet may help improve clinical conditions contributing to the development of IHD such as hypertension, diabetes, hyperlipidaemia, overweight and obesity[1,3–5].

Nordic and European DG, including the Danish Official DG, are based on systematic literature reviews of studies concerning the association between food intake and different diet related diseases [6–8]. In addition, the baseline part of the present study has shown that higher adherence to the Danish Official DG is associated with a more beneficial cardiometabolic risk profile in an adult Danish population [9]. However, DG targeting one specific nutrition-related lifestyle disease such as IHD may be even more effective in prevention of IHD than guidelines targeting all relevant nutrition-related diseases.

The evidence-base for the Danish official DG has found a convincing or probable causal relationship between the intake of fish, fruit and vegetables, whole grains and dietary fibre and substitution of saturated fat with polyunsaturated fat, and a reduced risk of IHD and overall cardiovascular diseases (CVDs) [6]. This is supported by recent results from meta-analyses that provide strong evidence that replacing saturated fat (SFA) with polyunsaturated fat (PUFA) and whole grains benefit cardiovascular health [10–12].

It is well established that when individuals change their dietary intake of specific foods, they primarily change their dietary composition rather than their total energy intake [13]. Therefore it is important not only to evaluate the single foods or nutrients which are consumed but also to look at the replaced foods or nutrients [14].

By emphasising specific food and macronutrient replacements we have previously shown in this randomised controlled trial (RCT) that targeted substitution DG was effective in changing the dietary composition of the overall diet towards being more cardio-protective [Arentoft et al. submitted]. However, the effect of targeted Substitution DG on IHD risk factors is still unknown. Therefore, the objective of this study was to examine the short- and long-term effects of applying targeted Substitution DG on IHD risk factors in an adult Danish population with a minimum of one self-reported risk factor of IHD.

Methods

Study design

The methodology of the study has been described previously [Arentoft et al. submitted]. In short, a six-month single-blinded, parallel RCT was conducted in a real-life setting, with a six-month follow-up. At baseline, participants were assigned to one of three study groups I) food-based Substitution DG II) food-based Danish Official DG, or III) a habitual diet.

Short- and long term effects of the DGs were defined as the changes in IHD risk factors from baseline to six months (end of the intervention) and from baseline to 12 months (follow-up), respectively.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by The Capital Region of Denmark Ethics Committee (Journal no. H-1-2013-110) and the Danish Data Protection Agency (Journal no. 2013-54-0571). Written informed consent was obtained from all study participants and they received a small remuneration of around 34 GBP for their participation in the study. The study was registered at ClinicalTrials.gov (registry name “Diet

and Prevention of Ischemic Heart Disease: a Translational Approach (DIPI)", ID no. NCT02062424).

Study participants

Potential participants were identified using a unique personal identification number assigned to all Danish citizens in the Civil Registration System [15].

The inclusion criteria were age between 30 and 65 years, and a minimum of one self-reported risk factor for IHD, i.e. overweight or obesity (BMI ≥ 25), waist circumference ≥80 cm for women and ≥94 cm for men, and/or physical inactivity defined as being moderately physically active in leisure time for 15 minutes or less per week.

The exclusion criteria were current smoking, pregnancy or plans to become pregnant within 12 months, breastfeeding, history of cardiovascular disease (CVD), type-2 diabetes, chronic disease/disorders that could affect the results of the study (the chronic diseases that the subjects reported were evaluated by the physician in charge), drug abuse within the last 12 months, regular alcohol consumption >21 units/week for men or >14 units/week for women, allergies or intolerance of the food groups included in the DG, consumption of dietary supplements with high doses of nutrients that could have a potential effect on IHD risk factors (e.g. fish oils) and/or no access to a computer and internet.

Randomisation and intervention

After the baseline examination, the study participants were randomly assigned to one of the three study groups.

The guidelines given to the participants in the targeted Substitution DG group focused on five of the ten Danish Official DG (**Table 1**), where the scientific evidence for a relationship between the dietary factor and an IHD outcome was found convincing or probable [6].

The guidelines given to the participants in the Official DG group included ten guidelines on food, beverages, and physical activity (**Table 1**). The Danish Official DG were updated based on a systematic literature review and knowledge regarding Danish food habits and relevant nutrition-related diseases [6].

Information about the DG was given via a leaflet with the respective guidelines and via a homepage (www.dipi.food.dtu.dk). Bi-weekly e-mails with two new recipes for each of the two study groups were sent out to the participants during the intervention period. In the habitual diet group the participants were also sent an email every second week, including a 'thank you for still participating' greeting.

Assessment of ischemic heart disease risk factors

Blood samples

Fasting blood samples from venipuncture were analysed for concentrations of triglycerides (TAG), total cholesterol, high density lipoprotein-cholesterol (HDL-cholesterol), high-sensitivity C-reactive protein (hsCRP), glucose, haemoglobin A_{1c} (HbA_{1c}) and insulin. The blood samples were collected and handled according to hospital routines. TAG, total cholesterol, HDL-cholesterol and glucose were measured in plasma by Reflection Spectroscopy by 540 nm, and hsCRP was measured in plasma by Reflection Spectroscopy by 660 nm (Apparatus Vitros 5.1 FS, Ortho-Clinical Diagnostics, Bridgend Pencoed, United Kingdom). HbA_{1c} was measured in plasma with High Performance Liquid Chromatography (HPLC) (D-100, Bio-Rad, Copenhagen, Denmark). Fasting plasma insulin was measured using the sandwich ELISA analysis principle (ADVIA Centaur XP,

Siemens, Ballerup, Denmark). The within-run variations (CV%) for the biochemical measurements were 0.7- 11%. Very low density lipoprotein-cholesterol (VLDL-cholesterol) was calculated from TAG using the equation; plasma VLDL-cholesterol = plasma TAG x 0,45 and low density lipoprotein-cholesterol (LDL-cholesterol) was calculated using The Friedewald Equation [16].

The homeostatic model assessment (HOMA) was used to estimate insulin resistance (HOMA-IR). HOMA-IR was calculated using the formula: HOMA-IR = [glucose (nmol/L) * insulin (mU/mL)/22.5], using fasting values [17].

Blood pressure and heart rate

Seated blood pressure (BP) and heart rate (HR) were measured using an electric sphygmomanometer according to standardized procedures.

Anthropometric measurements (height, weight, and waist circumference)

Height was measured to the nearest 0.5 cm on a wall mounted Stadiometer (SECA, Hamburg, Germany). Fasting body weight in kg and trunk fat was registered on a fat analysis weight (Tanita BC 418 MA, Tokyo, Japan). Waist- and hip circumference were measured twice, with an anthropometric tape (SECA 201, Hamburg, Germany) and the average was reported.

Body mass index (BMI) was defined as weight in kg divided by squared height in meters (kg/m^2).

Background questionnaires

Lifestyle questionnaires were used to obtain information about the participant's education level and the level of physical activity at leisure time.

Statistical method

Two multiple linear regression models were used to calculate changes in IHD risk factors in the group receiving the targeted Substitution DG and the group receiving the Official DG, when

compared to the Habitual diet group from baseline to six months and from baseline to 12 months. Model 1 was adjusted for baseline levels of the outcome variable, and model 2 was further adjusted for sex, age group (<50 and ≥50) and BMI group (18.5-25 = Normal weight, >25-30 = Overweight, >30 = Obese). In Model 2 we additionally tested for statistically significant interactions between the effects of intervention group and sex, intervention group and age group, and intervention group and BMI group. These interactions were tested to investigate whether the intervention had different effects for men and women, for participants above or below the 50 years of age or for normal weight, overweight or obese study participants. If an interaction was statistically significant, separate results according to the level of the effect modifier were provided.

To check the model assumptions, the standardized residuals of the final models were examined for normality, variance homogeneity and linearity.

The statistical analyses were carried out using RStudio (Version 0.99.441 – © 2009-2015 RStudio, Inc.). Statistical significance was established at $p < 0.05$.

Results

Baseline characteristics

A flow chart of the study has been presented previously [Arentoft et al. submitted]. A total of 222 participants were enrolled into the study. Three participants dropped out of the study before randomisation and were excluded. Baseline characteristics of the remaining 219 study participants by intervention group are presented in **Table 2**.

Retention

Altogether, 203 study participants completed the examination after six months, corresponding to a retention rate of 93%, and 196 study participants completed the examination after 12 months.

Medication users

Study participants taking cholesterol lowering (at baseline n=5, at 6 month n=7 and at 12 month n=3) and/or BP-lowering (at baseline n=3, at six month, n=2 and at 12 month n=6) medications were excluded from the statistical analysis including lipid biomarkers and BP, respectively.

Differences in IHD risk factors from baseline to six and 12 months

Table 3 and Table 4 show the between group differences in IHD risk factors from baseline to six months and from baseline to 12 months, respectively.

When compared to the habitual diet, no overall differences in changes in cardiometabolic risk factors were found in neither of the two DG groups. A statistically significant larger decrease in waist circumference (-4.41 cm, 95%CI: -7.93, -0.88cm) was found from baseline to 12 month in obese study participants receiving the Official DG, when compared to the Habitual diet.

Discussion

In the present study no overall differences in changes in IHD risk factors were found in either the group receiving the Substitution DG or the group receiving the Official DG, when compared to the habitual diet.

The results are in line with the findings of a recently published six-month RCT with a 12 months follow up by Jenkins et al. [18]. The study investigated the effect of dietary advises with and without food provision on weight loss and CVD risk factors and included 919 healthy overweight adult men and women [18]. The study participants were randomised to four groups, all receiving the Health Canada´s Food Guide. No additional advice was given to the control group. The first intervention arm out of three received dietary advise due to the DASH diet and the Portfolio diet, the second intervention arm were weekly provided with foods reflecting this advice, and the third intervention

arm were provided with food and advice. Comparable with the previously found results on dietary intake (Arentoft et al. Submitted), Jenkin et al. also observed an increase in whole grain intake in all three groups when compared to the control group. However, Jenkins et al. only observed increases in intake of other recommended foods in the groups provided with foods when compared to the control group. In addition and in line with the results of the present study, Jenkin et al. found no difference in CVD risk factors from baseline to six months or from baseline to 18 months in either of the three intervention groups when compared to the control group.

Several other RCTs conducted in a real life setting with a free living population comparable to the present study and the study by Jenkins et al. have investigated the effect of national DG and different diets such as the New Nordic Diet and the Mediterranean diet on CVD risk factors [19–21]. In one RCT, Reidlinger et al. found a beneficial effect of adherence to the United Kingdom (UK) DG, broadly similar to the Official DG, when compared to a traditional British diet on selected CVD risk factors [20]. Similarly, Estruch et al. found beneficial effects on CVD risk factors of two different Mediterranean diets (supplemented with extra olive or nuts) when compared to a low-fat diet [19].

Differences between the results of the studies by Reidlinger et al. and Estruch et al. and the present study could be due to variations in the study design. In the present study adherence to the DG were sought achieved through dietary advice about the DG on leaflets, a homepage including information on the DG and recipes and biweekly e-mails with new recipes for inspiration and motivation. This communication form was chosen to reflect how information of DG is normally transmitted in real-life. In contrast, adherence to both the UK DG investigated by Reidlinger et al. and the Mediterranean diet investigated by Estruch et al. were achieved by dietary advice provided by face-to-face meetings with a dietitian, which may be assumed to be more a more motivating information medium for the study participants [19,20]. In the study by Jenkins et al. on the other hand, the dietary

advice was provided through 20-30 minutes of telephone interviews administered weekly in the first months and monthly in the last five months of the intervention [18].

Contrary to the present study, the participants in the studies by Reidlinger et al. and Estruch et al. were also provided with key foods of the UK DG or the Mediterranean diet. Adherence to DG or specific diets may be better when study participants get the "new" key foods free of charge, instead of having to change shopping behaviour to obtain them themselves. This was also shown in the study by Jenkins et al. where the adherence to dietary advice were higher in the groups provided with foods reflecting the dietary advice given [18].

The baseline health status of the study participants may be of significance when measuring risk factors for CVD. In the present study, we recruited participants with a minimum of one self-reported risk factor for IHD, but even though the majority of the study participants were overweight and obese and had elevated waist circumference, the study participants were generally healthy, non-smoking, and not taking blood pressure- or hyperlipidaemia medication. This is similar to the populations of the studies by Jenkins et al. and Reidlinger et al. which also included healthy adult men and women. [18,20,21]. However, in contrast, the RCT by Estruch et al. included middle-aged men and women with high cardiovascular risk [19].

The fact that we did not observe any overall differences in change in IHD risk factors in the two DG groups when compared to the Habitual diet could also be due to the study participants baseline/habitual dietary intake of key food and nutrients included in both sets of DG. When compared to the habitual diet, we found the largest dietary changes in intake of whole grains, fish and SFA in both DG groups (Arentoft et al. submitted). An increased intake of both wholegrains and fish including high amounts of polyunsaturated fatty acids has been associated with reduced risk of IHD [6]. In addition, there is strong evidence that consuming PUFA in place of SFA reduces IHD [10,12,22].

However, the study participant's median baseline/habitual intake of whole grains and fish was already relatively high [20, 21 and Arentoft et al. Submittetd] and almost reached the recommended 75g/10MJ/ day and 50g/10MJ/day, respectively [6]. By comparison, the mean baseline intake of whole grains in in the studies by Reidlinger et al. and Jenkins et al. was only 1.4 -1.5 servings/ day (1 serving 30 g) ~ 43 - 45 g/day [18,20].

Our study has some limitations. Ensuring adherence to DGs is difficult in a long-term RCT, both because of prohibitive costs and of what can be imposed on the study participants in a long-term trial. However, the present study does provide evidence for the effects of *advising* two different sets of DGs on IHD risk factors; one set targeting primary prevention of IHD and focused on specific substitutions and one set of DGs focusing on nutrition-related diseases relevant in Denmark with a wording more traditional in the European and Nordic countries, namely the Danish Official DG.

Furthermore, the participants resided in areas of Greater Copenhagen with a relatively higher socioeconomic status and level of education compared to the general Danish population [23].

A strength of this study is the real-life setting where participants were free-living and empowered to modify their dietary pattern.

In conclusion, neither the Substitution DG targeting prevention of IHD nor the Danish Official DG showed any overall effects on IHD risk factors, when compared with the habitual diet, in an adult Danish population with a minimum of one self-reported risk factor for IHD in a free-living dietary advice RCT.

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Conflict of Interest

The authors declare no conflicts of interest.

Authorship

JLA, KO and IT formulated the research question; JLA, CH and IT contributed to the study design; JLA performed the statistical analyses, JLA, CH, RA, EWA, KO and IT were all involved in the interpretation of the data and critical revision of the manuscript; JLA drafted the manuscript and all authors approved the final version.

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Arentoft JL, Hoppe C, Andersen R, Andersen EW, Landberg R, Overvad K, Tetens I, Dietary effects of targeted substitution dietary guidelines in an adult Danish population: The

Table 1: The Substitution dietary guidelines and the Danish Official dietary guidelines

Substitution dietary guidelines	Official dietary guidelines
	Eat a variety of foods, but not too much, and be physically active
Eat fruit instead of candy and cake	
Eat coarse vegetables instead of fine vegetables†	Eat fruits and many vegetables
Eat fish instead of red meet	Eat more fish
Eat wholegrain products instead of products with no wholegrain grains	Choose whole grains
	Choose lean meats and cold meats
	Choose low-fat dairy products
Eat unsaturated fat instead of saturated fat	Eat less saturated fat
	Eat foods with less salt
	Eat less sugar
	Drink water

† Vegetables are classified from type of food groups (e.g. all types of cabbage, rooted vegetables and onions are classified as coarse vegetables and all vegetables with a high water content like tomatoes and salad are classified as fine vegetables)

Table 2 Baseline characteristics of the study participants by randomised intervention group (n=219)

Participants characteristics	HAB (n=73)		SUB (n=74)		OFF (n=72)	
	Median	(p25-p75)	Median	(p25-p75)	Median	(p25-p75)
Age (years)	51	(42, 55)	51	(42, 57)	53	(45, 58)
Women, % (n)	59	(43)	58	(43)	60	(43)
Weight (kg)	85.2	(71.8, 90.8)	82.2	(74.2, 88.8)	80.7	(70.5, 91.8)
BMI†	26.0	(24.0 , 29.3)	27.0	(25.6 , 29.2)	26.8	(24.6, 29.4)
Weight status†:						
Normal weight, % (n)	33	(24)	22	(16)	28	(20)
Overweight, % (n)	51	(37)	56	(42)	51	(37)
Obese, % (n)	16	(12)	22	(16)	21	(15)
Waist circumference (cm)	92.3	(85.7, 99.4)	92.3	(86.8, 98.2)	94.1	(83.1, 99.2)
Hip circumference (cm)	106.9	(103.7, 112.4)	107.2	(104.5, 114.5)	107.1	(102.8, 112.7)
Waist/hip-ratio	0.85	(0.80, 0.92)	0.85	(0.81, 0.90)	0.84	(0.79, 0.91)
Body fat (%)	33.4	(25.0, 37.7)	34.6	(24.5, 37.9)	31.5	(25.7, 38.9)
Trunk fat (%)	31.9	(26.5, 36.4)	33.7	(25.6, 36.2)	31.4	(27.4, 37.6)
Systolic blood pressure (mm Hg) ‡	128	(118, 138)	132	(120, 142)	128	(117, 137)
Diastolic blood pressure (mm Hg) ‡	79.8	(74.8, 87.0)	80.7	(75.6, 89.8)	80.0	(74.6, 86.6)
hsCRP (mg/L)	1.6	(0.5, 3.6)	1.4	(0.6, 3.2)	1.4	(0.5, 2.7)
Lipid biomarkers ¨						
Total cholesterol	5.2	(4.7, 5.9)	5.4	(4.8, 6.3)	5.2	(4.6, 5.9)
LDL-HDL ratio	2.7	(1.7, 3.1)	2.5	(1.8, 3.0)	2.1	(1.6, 2.6)
LDL cholesterol (mmol/L)	3.1	(2.8, 3.8)	3.3	(2.6, 4.2)	3.1	(2.5, 3.7)
HDL cholesterol (mmol/L)	1.4	(1.1, 1.7)	1.5	(1.1, 1.7)	1.5	(1.2, 1.8)
VLDL cholesterol (mmol/L)	0.4	(0.3, 0.7)	0.5	(0.4, 0.7)	0.5	(0.3, 0.6)
TAG (mmol/L)	1.0	(0.7, 1.6)	1.1	(0.8, 1.6)	1.0	(0.8, 1.3)
Glycaemic biomarkers ^						
Glucose (mmol/L)	5.5	(5.2, 5.8)	5.5	(5.2, 5.7)	5.4	(5.2, 5.8)
HbA1c (%)	5.0	(4.8, 5.1)	5.0	(4.9, 5.2)	5.0	(4.8, 5.2)
HOMA-IR	2.1	(1.6, 3.0)	1.9	(1.4, 3.0)	2.0	(1.3, 2.9)
Insulin (pmol/L)	60.5	(46.3, 81.8)	57.5	(41.3, 81.5)	57.0	(37.0, 80.5)

Educational level						
Primary school or high school, % (n)	22	(16)	26	(19)	29	(21)
Associate degree, % (n)	11	(8)	8	(6)	6	(4)
Undergraduate school, % (n)	37	(27)	42	(31)	40	(29)
Graduate school, % (n)	30	(22)	24	(18)	25	(18)

Abbreviations: HAB, habitual diet; SUB, Substitution dietary guidelines; OFF, Official dietary guidelines; BMI, Body Mass Index; hsCRP, high-sensitivity C-reactive protein; LDL, low density lipoprotein-cholesterol; HDL, high density lipoprotein-cholesterol; VLDL, very low density lipoprotein-cholesterol; TAG, triglycerides; estimated average glucose, eAG ; HbA1c , haemoglobin A_{1c}; HOMA-IR, homeostatic model of insulin resistance

†BMI is calculated as weight in kilograms divided by the square of height in meters (kg/m2). 18.5-25 = Normal weight, 25-30 = Overweight, >30 = Obese.

‡ all; n = 216, Substitution DG; n=72, Official DG; n=72, Habitual diet; n=72, after exclusion of those using blood pressure lowing medication

¨ n=214 at baseline after exclusion of those using cholesterol lowering medication

^ n=218 at baseline as it was not possible to draw enough blood for the glycaemic biomarkers analysis

Table 3 Between group differences in cardiometabolic risk factors from baseline to the end of the intervention (6 month).

	Model 1†				Model 2‡			
	SUB vs. HAB		OFF vs. HAB		SUB vs. HAB		OFF vs. HAB	
	Mean between group difference, 95%CI		Mean between group difference, 95%CI		Mean between group difference, 95%CI		Mean between group difference, 95%CI	
Weight (kg)	-0.41	(-1.40, 0.59)	-0.70	(-1.69, 0.29)	-0.40	(-1.40, 0.60)	-0.67	(-1.67, 0.32)
BMI¨	3.88	(-4.25, 12.02)	2.46	(-5.66, 10.59)	-0.13	(-0.47, 0.20)	-0.24	(-0.58, 0.09)
Waist circumference (cm)	-0.17	(-1.53, 1.19)	0.28	(-1.07, 1.64)	-0.29	(-1.62, 1.05)	0.11	(-1.22, 1.45)
Normal weight					-0.27	(-2.75, 2.20)	-1.07	(-3.44, 1.30)
Overweight					-1.14	(-2.93, 0.64)	1.13	(-0.67, 2.93)
Obese					2.45	(-0.67, 5.57)	-0.98	(-4.19, 2.24)
Hip circumference (cm)	-0.45	(-1.52, 0.62)	-0.04	(-1.10, 1.03)	-0.61	(-1.64, 0.41)	-0.09	(-1.11, 0.93)
Waist/hip-ratio	0.004	(-0.008, 0.02)	0.002	(-0.01, 0.01)	0.003	(-0.009, 0.02)	-0.00009	(-0.01, 0.01)
Body fat (%)	0.20	(-0.44, 0.84)	-0.04	(-0.68, 0.60)	0.08	(-0.54, 0.70)	-0.10	(-0.72, 0.5)
Trunk fat (%)	0.23	(-0.54, 0.99)	-0.08	(-0.85, 0.68)	0.12	(-0.62, 0.87)	-0.12	(-0.86, 0.62)
Systolic blood pressure (mm Hg) ^	1.39	(-1.80, 4.59)	-1.00	(-4.16, 2.16)	1.25	(-1.95, 4.45)	-1.28	(-4.46, 1.89)
Diastolic blood pressure (mm Hg) ^	1.57	(-0.13, 3.28)	-0.45	(-2.14, 1.24)	1.37	(-0.32, 3.05)	-0.73	(-2.40, 0.93)
hsCRP (mg/L) §	0.23	(-0.18, 0.65)	-0.03	(-0.44, 0.39)	0.18	(-0.23, 0.58)	-0.04	(-0.44, 0.36)
Lipid biomarkers ‖								
Total cholesterol	-0.11	(-0.30, 0.07)	-0.06	(-0.24, 0.11)	-0.12	(-0.29, 0.06)	-0.08	(-0.26 0.10)
LDL-HDL ratio	0.009	(-0.14, 0.16)	0.03	(-0.12, 0.18)	0.007	(-0.14, 0.16)	0.04	(-0.11, 0.19)
LDL cholesterol (mmol/L)	-0.07	(-0.23, 0.08)	-0.02	(-0.18, 0.13)	-0.08	(-0.23, 0.08)	-0.03	(-0.19, 0.12)
HDL cholesterol (mmol/L)	-0.001	(-0.06, 0.06)	-0.01	(-0.06, 0.05)	-0.001	(-0.06, 0.06)	-0.01	(-0.07, 0.05)
VLDL cholesterol (mmol/L)	-0.04	(-0.12, 0.05)	-0.02	(-0.10, 0.06)	-0.04	(-0.12, 0.04)	-0.02	(-0.10, 0.07)
TAG (mmol/L)	-0.07	(-0.26, 0.11)	-0.04	(-0.22, 0.15)	-0.09	(-0.28, 0.10)	-0.03	(-0.22, 0.15)
Glycaemic biomarkers¶								
Glucose (mmol/L)	0.08	(-0.04, 0.19)	0.04	(-0.08, 0.16)	0.06	(-0.05, 0.18)	0.03	(-0.08, 0.15)
HbA_{1c} (%)	0.01	(-0.07, 0.08)	0.01	(-0.06, 0.09)	-0.001	(-0.08, 0.07)	0.01	(-0.07, 0.08)
HOMA-IR	-0.05	(-0.43, 0.33)	-0.02	(-0.40, 0.35)	-0.06	(-0.44, 0.32)	-0.04	(-0.42, 0.34)
Insulin (pmol/L)	-2.23	(-11.99, 7.54)	-1.67	(-11.34, 8.01)	-2.56	(-12.37, 7.25)	-2.29	(-12.00, 7.42)

Abbreviations: HAB, Habitual diet; SUB, Substitution dietary guidelines; OFF, Official dietary guidelines; BMI, Body Mass Index; BP, blood

pressure; hsCRP, high-sensitivity C-reactive protein; LDL, low density lipoprotein-cholesterol; HDL, high density lipoprotein-cholesterol; VLDL, very low density lipoprotein-cholesterol; TAG, triglycerides; HbA_{1c}, haemoglobin A_{1c}; HOMA-IR, homeostatic model of insulin resistance

† simple linear regression models adjusted for baseline intake of the outcome variable

‡ multiple linear regression models adjusted for baseline intake of the outcome variable, sex, age group (<50 and ≥50), BMI group (18.5-25 = Normal weight, >25-30 = Overweight, >30 = Obese) and interactions between intervention group and sex, intervention group and age group, and intervention group and BMI group

¨ BMI is calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

^ n=216 at baseline, and n= 201 at 6 month after exclusion of those using BP-lowering medication

§ n= 204 at baseline, and n=196 at 6 month due to lack in biochemical analysis of hsCRP

‖ n=214 at baseline, and n=196 at 6 month after exclusion of those using cholesterol lowering medication

¶ n=218 at baseline, and 201 at 6 month as it was not possible to draw enough blood for the glycaemic biomarkers analysis

Table 4 Between group differences in cardiometabolic risk factors from baseline to follow-up (12 month).

	Model 1†				Model 2‡			
	SUB vs. HAB Mean between group difference, 95%CI		OFF vs. HAB Mean between group difference, 95%CI		SUB vs. HAB Mean between group difference, 95%CI		OFF vs. HAB Mean between group difference, 95%CI	
Weight (kg)	-0.23	(-1.43, 0.97)	-0.52	(-1.71, 0.67)	-0.19	(-1.40, 1.03)	-0.47	(-1.68, 0.73)
BMI¨	-0.07	(-0.47, 0.33)	-0.19	(-0.59, 0.22)	-0.06	(-0.47, 0.35)	-0.17	(-0.58, 0.23)
Waist circumference (cm)	-0.92	(-2.33, 0.50)	-0.05	(-1.46, 1.36)	-0.92	(-2.33, 0.48)	-0.08	(-1.49, 1.32)
Normal weight					-0.94	(-3.55,1.67)	-0.43	(-2.88, 2.02)
Overweight					-1.31	(-3.15, 0.53)	1.27	(-0.58,3.13)
Obese					0.28	(-3.06, 3.63)	-4.41	(-7.93,-0.88)
Hip circumference (cm)	0.04	(-1.14, 1.21)	-0.23	(-1.40, 0.94)	-0.07	(-1.24, 1.11)	-0.27	(-1.43, 0.90)
Normal weight					0.06	(-2.15, 2.27)	-1.20	(-3.27, 0.86)
Overweight					-0.8	(-2.34, 0.76)	0.46	(-1.10, 2.03)
Obese					2.27	(-0.56, 5.11)	-1.05	(-3.99, 1.90)
Waist/hip-ratio	-0.008	(-0.02, 0.004)	0.001	(-0.01, 0.01)	-0.007	(-0.02, 0.005)	0.0008	(-0.01, 0.01)
Body fat (%)	0.61	(-0.18, 1.40)	-0.05	(-0.83 0.74)	0.59	(-0.20, 1.38)	-0.04	(-0.82, 0.74)
Trunk fat (%)	0.75	(-0.16, 1.66)	-0.05	(-0.95, 0.85)	0.75	(-0.16, 1.67)	-0.03	(-0.93, 0.88)
Systolic blood pressure (mm Hg) ^	-0.02	(-3.39, 3.35)	-2.53	(-5.89, 0.83)	-0.42	(-3.69 2.86)	-2.99	(-6.26, 0.28)
Diastolic blood pressure (mm Hg) ^	0.47	(-1.43, 2.37)	-1.00	(-2.90, 0.90)	0.24	(-1.63, 2.12)	-1.22	(-3.09, 0.65)
hsCRP (mg/L) §	0.11	(-0.41, 0.63)	-0.03	(-0.55, 0.50)	0.07	(-0.4, 0.60)	-0.03	(-0.56, 0.50)
Lipid biomarkers‖								
Total cholesterol	0.07	(-0.18, 0.31)	-0.14	(-0.38, 0.11)	0.07	(-0.17, 0.32)	-0.14	(-0.38, 0.10)
LDL-HDL ratio	0.05	(-0.14, 0.24)	-0.07	(-0.26, 0.12)	0.06	(-0.13, 0.26)	-0.06	(-0.25, 0.14)
LDL cholesterol (mmol/L)	0.10	(-0.11, 0.32)	-0.10	(-0.32, 0.11)	0.12	(-0.10, 0.33)	-0.10	(-0.31, 0.11)
HDL cholesterol (mmol/L)	0.03	(-0.04, 0.11)	-0.002	(-0.07, 0.07)	0.03	(-0.04, 0.10)	-0.01	(-0.08, 0.07)
VLDL cholesterol (mmol/L)	-0.05	(-0.15, 0.05)	-0.01	(-0.11, 0.09)	-0.06	(-0.16, 0.04)	-0.01	(-0.11, 0.08)
TAG (mmol/L)	-0.13	(-0.35, 0.09)	-0.03	(-0.25, 0.19)	-0.15	(-0.37, 0.07)	-0.04	(-0.25, 0.18)
Glycaemic biomarkers¶								
Glucose (mmol/L)	-0.005	(-0.13, 0.12)	0.05	(-0.07, 0.17)	-0.01	(-0.13, 0.10)	0.04	(-0.07, 0.16)
HbA_{1c} (%)	0.005	(-0.07, 0.08)	0.06	(-0.01, 0.13)	0.003	(-0.07, 0.08)	0.06	(-0.01,0.13)

HOMA-IR	-0.07	(-0.48, 0.34)	0.12	(-0.28, 0.52)	-0.08	(-0.48, 0.32)	0.11	(-0.29, 0.51)
Insulin (pmol/L)	-1.86	(-11.56, 7.84)	4.02	(-5.65, 13.69)	-2.09	(-11.66, 7.48)	3.58	(-5.95, 13.10)

Abbreviations: HAB, Habitual diet; SUB, Substitution dietary guidelines; OFF, Official dietary guidelines; BMI, Body Mass Index; BP, blood pressure; hsCRP, high-sensitivity C-reactive protein; LDL, low density lipoprotein-cholesterol; HDL, high density lipoprotein-cholesterol; VLDL, very low density lipoprotein-cholesterol; TAG, triglycerides; HbA_{1c}, haemoglobin A_{1c}; HOMA-IR, homeostatic model of insulin resistance

† simple linear regression models adjusted for baseline intake of the outcome variable

‡ multiple linear regression models adjusted for baseline intake of the outcome variable, sex, age group (<50 and ≥50), BMI group (18.5-25 = Normal weight, >25-30 = Overweight, >30 = Obese) and interactions between intervention group and sex, intervention group and age group, and intervention group and BMI group

¨ BMI is calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

^ n=216 at baseline, and n= 190 at 12 month after exclusion of those using BP-lowering medication

§ n= 204 at baseline, and n= 185 at 12 month due to lack in biochemical analysis of hsCRP

‖ n=214 at baseline, and n=193 at 12 month after exclusion of those using cholesterol lowering medication

¶ n=218 at baseline, and n=195 at 12 month as it was not possible to draw enough blood for the glycaemic biomarkers analysis

Appendix B: Table 1 to 3

Table 1: Overall Diet Quality Index (DQI) score, energy (MJ/d), diet composition (g/10 MJ/d), and energy contribution (E%) of macronutrients and dietary fibre (g/MJ) of the study population at baseline divided in tertiles of DQI score; medians (p10-p90), n=219.

Table 2: Within group differences in dietary data from baseline to 6 or 12 months; means and 95% CI, n=219 at baseline, n= 199 at 6 month and n= 186 at 12 months.

Table 3: Within group differences in IHD risk factors from baseline to 6 or 12 months; means and 95% CI, n=219 at baseline, n= 203 at 6 month and n= 196 at 12 months.

Table 1: Overall Diet Quality Index (DQI) score, energy (MJ/d), diet composition (g/10 MJ/d), and energy contribution (E%) of macronutrients and dietary fibre (g/MJ) of the study population at baseline divided in tertiles of DQI score; medians (p10-p90), n=219.

	T1		T2		T3	
DQI	3.2	(2.2-3.8)	4.5	(4.0-4.9)	5.8	(5.1-7.8)
Energy MJ/d	10	(7-13)	8	(6-12)	8	(5-12)
Diet composition (g/10 MJ/d)						
Bread and cereals	205	(130-281)	226	(170-307)	230	(136-338)
Whole grains	47	(24-72)	66	(40-98)	80	(40-142)
Meat and meat products	148	(70-261)	142	(67-266)	125	(50-232)
Fish and fish products †	24	(7-60)	47	(17-76)	92	(33-140)
Poultry and poultry products ‡	36	(7-123)	40	(2-105)	46	(14-102)
Fruit and vegetables	285	(138-543)	418	(209-611)	505	(237-849)
Fruit and food products	87	(30-202)	163	(59-323)	204	(53-435)
Vegetables and vegetables products	180	(85-335)	216	(113-382)	270	(134-468)
Vegetables, coarse¨	74	(24-178)	98	(27-183)	125	(51-259)
Vegetables, fine¨	99	(45-174)	112	(52-221)	127	(58-305)
Potatoes and potatoes products	65	(13-141)	48	(2-132)	50	(3-139)
Milk and milk products	233	(68-440)	257	(67-537)	225	(74-540)
Cheese and cheese products	40	(9-100)	46	(16-121)	49	(10-178)
Edible fats	33	(20-50)	32	(16-49)	26	(17-40)
Sugar and candy	52	(21-111)	36	(12-65)	25	(5-57)
Energy distribution						
Protein, E%	16	(14-19)	17	(14-22)	19	(15-23)
Fat, E%	36	(30-42)	36	(28-43)	34	(27-39)
SFA, E%	14	(12-17)	13	(11-16)	12	(9-14)
MUFA, E%	13	(11-17)	14	(11-17)	14	(10-16)
PUFA, E%	5	(4-6)	5	(4-7)	5	(4-7)
Carbohydrate, E%	45	(36-53)	45	(34-52)	43	(33-54)
Added sugar, E%	10	(5-18)	6	(3-11)	4	(2-9)
Dietary fibre, g/MJ	2	(2-3)	3	(2-3)	3	(2-4)
Alcohol, E% §	5	(1-12)	5	(1-13)	6	(2-15)

Abbreviations: T, tertile; g, gram; MJ, mega joule; E%, energy percentage; SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

† n=201 after exclusion of those who did not eat fish

‡ n=180 after exclusion of those who did not eat poultry

¨ Vegetables are classified from type of food groups (e.g. all types of cabbage, rooted vegetables and onions are classified as coarse vegetables and all vegetables with a high water content like tomatoes and salad are classified as fine vegetables).

§ n=194, after exclusion of those who did not drink alcohol.

Table 2 Within group differences in dietary data from baseline to 6 or 12 months; means and 95% CI, n=219 at baseline, n= 199 at 6 month and n= 186 at 12 months.

	Changes from baseline to 6 months						Changes from baseline to 12 months					
	Habitual diet		Substitution DG		Official DG		Habitual diet		Substitution DG		Official DG	
	Mean (95% CI)	Pr>|t|†	Mean (95% CI)	Pr>|t|†	Mean (95% CI)	Pr>|t|†	Mean (95% CI)	Pr>|t|†	Mean (95% CI)	Pr>|t|†	Mean (95% CI)	Pr>|t|†
Diet composition (g/10MJ/d):												
Bread and cereals	13 (-3, 28)	0.101	19 (2, 37)	**0.029**	7 (-8, 21)	0.365	2 (-14, 18)	0.813	12 (-4, 28)	0.143	4 (-14, 21)	0.688
Whole grains	-0.5 (-7, 6)	0.868	20 (9,30)	**0.001**	7 (1, 13)	**0.024**	-4 (-13, 4)	0.298	15 (6, 24)	**0.001**	10 (3,17)	**0.009**
Meat	-4 (-25,17)	0.697	-23 (-43, -3)	**0.024**	-17 (-38, 5)	0.122	7 (-16, 29)	0.546	-20 (-42, 2)	0.070	-16 (-37,5)	0.124
Fish	-0.3 (-13, 12)	0.956	13 (0.1, 26)	**0.048**	21 (5,38)	**0.013**	-8 (-21, 6)	0.248	17 (-1, 36)	0.068	24 (9, 38)	**0.002**
Poultry	2 (-11, 14)	0.783	3 (-17, 24)	0.739	4 (-9, 17)	0.504	-3 (-17, 11)	0.677	-12 (-33, 8)	0.237	8 (-9, 25)	0.350
Fruit	-12 (-30, 6)	0.197	18 (-16, 53)	0.294	20 (-13,54)	0.233	-1 (-29,27)	0.939	1 (-30, 32)	0.954	10 (-20, 40)	0.525
Vegetables	-16 (-48, 16	0.324	30 (-5, 64)	0.090	6 (-23, 36)	0.671	14 (-16, 44)	0.346	-3 (-56, 50)	0.904	9 (-22, 40)	0.557
Vegetables, coarse‡	-5 (-24, 14)	0.621	3 (-21, 28)	0.782	-6 (-25, 14)	0.547	8 (-12, 27)	0.421	-8 (-43,28)	0.674	3 (-18, 25)	0.755
Vegetables, fine‡	-2 (-25, 21)	0.870	27 (3, 50)	**0.028**	13 (-8, 34)	0.228	16 (-11,43)	0.244	7 (-18, 32)	0.580	6 (-11, 24)	0.465
Potatoes	3 (-14, 20)	0.694	-2 (-17,13)	0.813	7 (-16, 30)	0.537	4 (-12, 20)	0.635	-3 (-20, 15)	0.766	14 (-8, 37)	0.204
Milk	-7 (-62,47)	0.791	12 (-17, 41)	0.410	24 (-24, 71.0)	0.321	-28 (-75, 19)	0.240	17 (-8, 42)	0.172	-36 (-79, 7)	0.101
Cheese	15.4 (-0.1, 31)	0.052	0.1 (-11, 11)	0.993	-1.9 (-18, 15)	0.821	21 (1, 41)	**0.038**	0.7 (-12, 13)	0.913	18 (-3, 38)	0.090
Sucker and candy	-6.0 (-13, 1)	0.081	-4 (-11, 4)	0.334	-2.8 (-9, 3)	0.368	-6 (-13, 1)	0.085	2.0 (-5, 9)	0.574	-2 (-9, 5)	0.530
Dietary fibre, g/MJ/d	0.02 (-0.1, 0.2)	0.832	0.3 (0.2, 0.5)	**2.4 e^{-05}**	0.1 (-0.01, 0.2)	0.079	0.02 (-0.1, 0.1)	0.785	0.1 (-0.02, 0.3)	0.080	0.1 (-0.001, 0.3)	0.051
Energy MJ and E% of macronutrients												
Energy, MJ	-0.7 (-1.2, -0.2)	**0.001**	-0.8 (-1.3,-0.3)	**0.002**	-0.51 (-1.0, 0.01)	0.053	-0.9 (-1.4, -0.4)	**0.001**	-0.7 (-1.3, -0.2)	**0.005**	-0.5 (-1.0, 0.1)	0.090
Protein, E%	0.4 (-0.4, 1.1)	0.334	0.1 (-0.6, 0.8)	0.788	0.4 (-0.4, 1.1)	0.334	0.3 (-0.4, 1.1)	0.343	-0.01(-0.9, 0.9)	0.980	0.8 (0.04, 1.6)	**0.040**
Carbohydrate, E%	-0.02 (-1.5, 1.5)	0.982	2.0 (0.5, 3.6)	**0.012**	1.6 (-0.02, 3.2)	0.053	-0.5 (-1.9, 1.0)	0.500	0.9 (-0.7, 2.6)	0.264	0.1 (-1.5, 1.8)	0.872
Added sugar, E%	-0.7 (-1.7, 0.2)	0.113	-0.9 (-1.8,-0.1)	**0.042**	-0.9 (-1.8, -0.03)	**0.042**	-1.0 (-1.9. -0.03)	**0.043**	-0.2 (-1.1, 0.7)	0.647	-1.3 (-2.4, -0.2)	**0.025**
Total fat, E%	0.03 (-1.2, 1.3)	0.962	-1.8(-3.2, -0.4)	**0.011**	-1.0 (-2.3, 0.4)	0.169	0.08 (-1.2, 1.4)	0.902	-0.3 (-1.8, 1.2)	0.696	-0.6 (-1.9, 0.8)	0.384
SFA, E%	0.4 (-0.2, 1.1)	0.190	-1.4 (-2.1,-0.8)	**3.5 e^{-05}**	-0.6 (-1.3, 0.1)	0.088	0.3 (-0.4, 1.0)	0.356	-0.7 (-1.4, -0.03)	**0.041**	-0.8 (-1.4, -0.1)	**0.030**
MUFA, E%	-0.2 (-0.9, 0.8)	0.549	-0.6 (-1.4, 0.2)	0.134	-0.2(-0.9, 0.5)	0.595	-0.2 (-1.0, 0.7)	0.695	0.1 (-0.7, 0.9)	0.804	0.09 (-0.6, 0.8)	0.793
PUFA, E%	-0.02 (-0.3, 0.3)	0.878	0.3 (-0.1, 0.7)	0.184	0.05 (-0.3, 0.4)	0.745	-0.01 (-0.4, 0.3)	0.964	0.1 (-0.3, 0.5)	0.602	0.3 (-0.02, 0.7)	0.068
Alcohol, E%	-0.6 (-1.9, 0.7)	0.341	-0.1 (-1.0, 0.9)	0.897	-1.0 (-2.1, 0.1)	0.087	0.4 (-0.6, 1.4)	0.400	-0.8 (-1.7, 0.1)	0.092	-0.3 (-1.5, 0.9)	0.605

Abbreviations: Substitution DG, targeted substitution dietary guidelines; Official DG, Danish official dietary guidelines, MJ, mega joule; E %, energy percentage; SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

† pared t-test

‡ Vegetables are classified from type of food groups (e.g. all types of cabbage, rooted vegetables and onions are classified as coarse vegetables and all vegetables with a high water content like tomatoes and salad are classified as fine vegetables).

Table 3 Within group differences in IHD risk factors from baseline to 6 or 12 months; means and 95% CI, n=219 at baseline, n= 203 at 6 month and n= 196 at 12 months.

	Mean changes (95% CI) from baseline at six months						Mean changes (95% CI) from baseline at 12 months					
	habitual diet		substitution DG		official DG		habitual diet		substitution DG		official DG	
	Mean (95% CI)	Pr>|t|†	Mean (95% CI)	Pr>|t|†	Mean (95% CI)	Pr>|t|†	Mean (95% CI)	Pr>|t|†	Mean (95% CI)	Pr>|t|†	Mean (95% CI)	Pr>|t|†
Weight (kg)	-0.2 (-0.8, 0.5)	0.601	-0.6 (-1.2, 0.1)	0.071	-0.9 (-1.7, -0.04)	**0.040**	0.1 (-0.8, 0.9)	0.839	-0.2 (-1.2, 0.8)	0.644	0.3 (-0.4, 1.0)	0.385
BMI†	-0.1 (-0.3, 0.2)	0.581	-0.2 (-0.4, 0.03)	0.089	-0.3 (-0.6, -0.01)	**0.044**	0.1 (-0.1, 0.3)	0.4082	0.03 (-0.2, 0.3)	0.816	-0.1 (-0.4, 0.3)	0.611
WC (cm)	0.5 (-0.5, 1.4)	0.311	0.3 (-0.7, 1.2)	0.541	0.8 (-0.2, 1.8)	0.127	-1.7 (-2.5, -0.9)	**8.5e^{-05}**	-2.6 (-3.6, -1.6)	**9.3e^{-07}**	-1.7 (-2.9, -0.4)	**0.009**
HC (cm)	-1.5 (-2.2, -0.8)	**0.0001**	-1.9 (-2.8, -1.1)	**1.3e^{-05}**	-1.5 (-2.3, -0.8)	**0.0001**	-1.3 (-2., -0.6)	**0.001**	-1.3 (-2.2, -0.4)	**0.005**	-1.5 (-2.4, -0.6)	**0.001**
WC/HC-ratio	0.02 (0.01, 0.03)	**0.001**	0.02 (0.01, 0.03)	**0.0003**	0.02 (0.01, 0.03)	**6.1e^{-05}**	-0.01 (-0.01,0.003)	0.188	-0.01 (-0.02, -0.01)	**0.001**	-0.01(-0.01,0.01)	0.417
Body fat (%)	-0.41(-0.9, 0.04)	0.075	-0.2 (-0.6, 0.2)	0.289	-0.5 (-1.0,0.1)	0.085	-0.03 (-0.6, 0.5)	0.908	0.6 (0.1, 1.1)	**0.025**	-0.1 (-0.7, 0.6)	0.817
Trunk fat (%)	-0.4 (-0.9, 0.1)	0.099	-0.2 (-0.7, 0.3)	0.438	-0.5 (-1.2, 0.1)	0.123	-0.1(-0.6, 0.5)	0.780	0.7 (0.1, 1.3)	**0.030**	-0.1 (-0.9, 0.6)	0.735
Systolic BP (mm Hg)‡	-2.4 (-4.7, -0.1)	**0.043**	-1.2 (-3.8, 1.4)	0.366	-3.1 (-5.2, -0.9)	**0.006**	-2.1 (-4.8, 0.7)	0.145	-2.2 (-5.1, 0.7)	0.128	-3.8 (-5.9, -1.6)	**0.001**
Diastolic BP (mm Hg)‡	-1.4 (-2.6, -0.1)	**0.032**	0.1 (-1.2, 1.5)	0.832	-1.7 (-3.0, -0.4)	**0.010**	-1.9(-3.3, -0.5)	**0.010**	-1.4 (-3.1, 0.2)	0.079	-3.6 (-5.7, -1.4)	**4.4e^{-05}**
hsCRP (mg/L)¨	-0.1 (-0.4, 0.2)	0.396	-0.1 (-0.5, 0.3)	0.651	-0.2 (-0.6, 0.2)	0.301	-0.04 (-0.5, 0.4)	0.843	-0.02 (-0.4, 0.4)	0.936	-0.1 (-0.5, 0.3)	0.692
Lipid biomarkers^												
Total cholesterol	0.02 (-0.1, 0.2)	0.736	-0.1 (-0.3, 0.04)	0.121	-0.1 (-0.2, 0.03)	0.140	0.1 (-0.1, 0.3)	0.305	0.1 (-0.1, 0.3)	0.142	-0.1 (-0.3, 0.1)	0.331
LDL-HDL ratio	( )		( )		( )		( )		( )		( )	
LDL-c (mmol/L)	0.1 (-0.1, 0.2)	0.307	-0.1 (-0.2, 0.1)	0.376	-0.03 (-0.1, 0.1)	0.631	0.1 (-0.1, 0.3)	0.198	0.2 (0.02, 0.3)	**0.030**	-0.1 (-0.3, 0.1)	0.415
HDL-c (mmol/L)	-0.1 (-0.1, -0.03)	**0.001**	-0.1 (-0.1, -0.01)	**0.020**	-0.1 (-0.1, -0.02)	**0.004**	-0.1 (-0.1, -0.01)	**0.015**	-0.03 (-0.1, 0.02)	0.258	-0.1 (-0.1, 0.01)	0.095
VLDL-c (mmol/L)	0.04 (-0.003, 0.1)	0.070	-0.005 (-0.1, 0.1)	0.907	0.01 (-0.1, 0.1)	0.807	0.05 (-0.01, 0.1)	0.118	0.0001 (-0.1, 0.09)	0.955	0.02 (-0.1, 0.1)	0.515
TAG (mmol/L)	0.1 (-0.01, 0.2)	0.096	-0.02 (-0.2, 0.2)	0.858	0.02 (-0.1, 0.2)	0.772	0.1 (-0.02, 0.2)	0.095	-0.02 (-0.2, 0.2)	0.845	0.06 (-0.1, 0.2)	0.470
Glycaemic biomarkers§												
Glucose (mmol/L)	-0.2 (-0.3, -0.1)	**0.0001**	-0.1 (-0.2, -0.1)	**0.002**	-0.2 (-0.3, -0.1)	**0.001**	-0.01(-0.1, 0.1)	0.779	-0.01 (-0.1, 0.1)	0.804	0.04 (-0.04, 0.1)	0.304
HbA1c (%)	0.1 (0.1, 0.2)	**1.5e^{-06}**	0.2 (0.1, 0.2)	**4.1e^{-06}**	0.1 (0.1, 0.2)	**3.9e^{-05}**	0.2 (0.1, 0.2)	**2.9e^{-09}**	0.2 (0.1, 0.2)	**6.3e^{-09}**	0.2 (0.2, 0.3)	**1.2e^{-10}**
HOMA-IR	-0.03 (-0.3, 0.2)	0.800	-0.1 (-0.4, 0.2)	0.385	-0.04 (-0.3, 0.2)	0.740	-0.02 (-0.3, 0.2)	0.899	-0.2 (-0.5, 0.2)	0.395	0.1(-0.2, 0.4)	0.411
Insulin (pmol/L)	1.8 (-4.7, 8.3)	0.586	-1.6 (-9.7, 6.5)	0.691	0.6 (-6.2, 7.5)	0.853	-1.1 (-7.3, 5.1)	0.734	-5.6 (-14.9, 3.7)	0.234	3.7 (-3.3, 10.8)	0.293

Abbreviations: IHD, ischaemic heart disease; Substitution DG, targeted substitution dietary guidelines; Official DG, Danish official dietary guidelines, WC, Waist circumference; HC, Hip circumference; BP, blood pressure; hsCRP, high-sensitivity C-reaktive protein; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; VLDL-c, very low-density lipoprotein cholesterol; TAG, triglyceride; HbA_{1c}, haemoglobin A_{1c}; HOMA-IR, homeostatic model assessment insulin resistance

† pared t-test

‡ n=216 at baseline, n= 201 at 6 month and n= 190 at 12 month after exclusion of those using BP-lowering medication

¨ n= 204 at baseline, n=196 at 6 month and n= 185 at 12 month due to lack in biochemical analysis of hsCRP

^ n=214 at baseline, n=196 at 6 month and n=193 at 12 month after exclusion of those using cholesterol lowering medication

§ n=218 at baseline, 201 at 6 month and n=195 at 12 month as it was not possible to draw enough blood for the glycaemic biomarkers analysis