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DISSERTATION

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„Systematic reviews and meta-analyses in Nutritional Epidemiology:
effects of lifestyle modifications in health and disease“

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“Absence of evidence is not evidence of absence”

Carl Edward Sagan

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Abbreviations

ACC: American College of Cardiology

ACSM: American College of Sports Medicine

ADA: American Diabetes Association

AET: Aerobic exercise training

AHA: American Heart Association

AHEI: Alternate Healthy Eating Index

AICR: American Institute for Cancer Research

aMED: Alternate Mediterranean dietary pattern

BMI: Body Mass Index

BW: Body weight

CHD: Coronary heart disease

CRP: C-reactive protein

CT: Combined training

CVD: Cardiovascular disease

DASH: Dietary approaches to Stop Hypertension

DBP: Diastolic blood pressure

DGA: Dietary Guidelines for Americans

FM: Fat mass

FMD: Flow mediated dilatation

GFR: Glomerular filtration rate

GRADE: Grading of Recommendations Assessment, Development and Evaluation

HbA1c: Glycosylated haemoglobin

HDL-C: High-density lipoprotein cholesterol

HEI: Healthy Eating Index

HF: High fat

HGI: High glycaemic index

HGL: High glycaemic load

HP: High protein

ICAM-1: Intercellular adhesion molecule-1

IL-6: Interleukin-6

LBM: Lean body mass

LC: Low carbohydrate

LDL-C: low-density lipoprotein cholesterol

LF: Low fat

LGI: Low glycaemic index

LGL: Low glycaemic load

LOE: Level of evidence

LP: Low protein

MD: Mean difference

MED: Mediterranean diet

MUFA: Monounsaturated fatty acids

NCD: Non communicable diseases

NP: Normal protein

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PUFA: Polyunsaturated fatty acids

RCT: Randomized controlled trial

RR: Risk ratio

RT: Resistance training

SBP: Systolic blood pressure

SFA: Saturated fatty acids

T2D: Type 2 Diabetes mellitus

TC: Total cholesterol

TEC: Total energy content

TFA: Trans-fatty acids

TG: Triacylglycerols

TNF- α : Tumor-necrosis-factor-alpha

USDA: United States Department of Agriculture

VCAM-1: Vascular cell adhesion molecule-1

VO₂ max: Maximum oxygen uptake

WC: Waist circumference

WCRF: World Cancer Research Fund

WHO: World Health Organization

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1. Introduction

Lifestyle is a crucial factor in the prevention of non-communicable diseases (NCD). Large prospective cohort studies have shown that 60-75% of coronary events and 36% of cancer incidences can be explained by modifiable factors such as unhealthy diets, overweight, obesity, physical inactivity, smoking and inadequate alcohol intake [1-3]. Next to cholesterol-lowering drug therapies, changes in lifestyle remain a key component in the secondary prevention of cardiovascular disease (CVD) [4, 5]. On the other hand, there is currently only limited or insufficient evidence for a preventive effect of lifestyle interventions in cancer survivors [6, 7]. An adequate approach to clarify inconclusive data and knowledge in the field of public health nutrition might be to systematically review and meta-analyse large amounts of published data in order to further our understanding in the interplay between lifestyle, diet and health [8].

The first part of the introductory chapter of this cumulative thesis will briefly describe paramount indicators of chronic diseases (CVD, cancer and type 2 diabetes mellitus (T2DM)), i.e. mortality and incidence rates as well as the prevalence of the underlying risk factor overweight/obesity. Subsequently, a summary of dietary and physiological risk factors, as well as different prevention strategies for chronic diseases (including a critical review on adherences of lifestyle recommendations provided by international authorities, such as the American College of Cardiology (ACC), the American Heart Association (AHA), the American College of Sports Medicine (ACSM), the American Diabetes Association (ADA), the United States Department of Agriculture (USDA), the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) will be given. The final section of the introduction will emphasize the relevance of

systematic reviews and meta-analyses in evidence-based medicine, public health as well as general methodological aspects of systematic reviews. The second chapter will focus on the aims of the present thesis, while the third chapter will report on the published manuscripts of this cumulative dissertation by reproducing the abstracts of each manuscript. Chapter four will provide a critical discussion of the results in a scientific context as well as a comparison of findings of currently recommended lifestyle guidelines. A detailed description of the scientific background, the methodological aspects and the discussion of the individual findings are given in the corresponding manuscripts. The final chapter will outline the most important conclusions provided by the studies together with future aspects regarding the topics of this thesis.

1.1 Global Mortality rates: CVD, type 2 diabetes mellitus and cancer

In 2013, the number of deaths worldwide and throughout all age groups amounted to nearly 55 million people, with 70% of them caused by NCD [9]. 32% of fatalities were caused by cardiovascular or circulatory diseases (15% by ischemic heart disease, 12% by stroke and approximately 3% by T2DM) followed by cancer mortality with 15% (overall 3% of all cases died from lung cancer). Since 1990, there was a significant worldwide increase in the age-standardized death rates for CVD and T2DM with a simultaneous significant decrease in the death rates for neoplastic disease [9].

1.2 Global prevalence: CVD, type 2 diabetes mellitus and cancer

Data from 11 European countries (Survey of Health, Ageing and Retirement), the United States (Health Retirement Survey) and the United Kingdom (English Longitudinal Study

of Aging) indicate that the United States has the highest prevalence of heart disease and stroke in both males and females [10]. Diabetes is associated with a three times higher CVD mortality rate compared to non-diabetic participants [11] and represents one of the most serious and pressing current health problems, with an estimated prevalence of 350 million cases worldwide, according to the World Health Organization (WHO) in 2013 [12]. 90-95% of diabetes cases were assigned to T2DM [12]. In spite of a decrease in death due to neoplastic disease, cancer remains a tremendous health problem. According to estimations of the International Agency for Research on Cancer, the 5-year global cancer prevalence amounted to ~28.8 million in 2008. Developed countries that comprise only one-sixth of the world's population account for half of the global cancer prevalence. With respect to localization, the most prevalent cancer worldwide continues to be breast cancer. Prostate cancer is the most common neoplastic disease in the United States, Oceania as well as Western and Northern Europe. In southern Asia and sub-Saharan Africa, cervix cancer is the most prevalent neoplasm, whereas in Eastern Asia gastric cancer is the most frequent form [13].

1.3 Prevalence and consequences of overweight/obesity and associated diseases

Obesity is a major risk factor for CVD, diabetes and cancer as well as other chronic diseases (e.g. depression, chronic kidney disease and osteoarthritis). Cardiovascular mortality is the main cause of death in overweight and obese patients [14-18].

According to the WHO, at least one third of adults were either overweight or obese (Body Mass Index (BMI) ≥ 25 kg/m²) in 2008 with nearly 300 million women and 205 million

men being obese. This indicates a tremendous worldwide increase in the prevalence of obesity since 1980 ($\text{BMI} \geq 30 \text{ kg/m}^2$) [19]. While in the western world, the prevalence data seem to have stabilized, the prevalence of adult obesity in developing countries is still increasing [20]. According to the United Nations, in 2014 more than half of the world's population lived in urban areas [21]. Urbanization is often associated with a sedentary lifestyle and an unhealthy diet, thus paving the way to obesity [22, 23]. In the United States, approximately 35% of US adults (≥ 20 years of age) were considered to be obese in 2011/2012 [24]. In Europe, the prevalence of obesity ranged from 6.2% to 36.5% in women and from 4.0% to 28.3% in men. The highest obesity rates ($>25\%$) could be observed in Italy, Spain, Poland and the Czech Republic, Romania (only women), Albania (only women) and Portugal (only women) [25]. The world's highest prevalence of obesity could be detected in Kuwait (58.6% for women and 43.3% for men), Qatar (54.7% for women and 44% for men) and Samoa (69.1% for women and 45.9% for men) [20]. Obesity is a multifactorial disease defined as excess body weight (BW) for height. This etiologically complex phenotype is primarily associated with excess body fat (mainly visceral fat), that can manifest metabolically [26]. The European Prospective Investigation into Cancer and Nutrition (EPIC) cohort including more than 500,000 people has shown that a BMI of 24.3 kg/m^2 for women and 25.3 kg/m^2 for men was associated with the lowest mortality risk [27]. In 2010, overweight and obesity caused an estimate of 3.4 million deaths worldwide, equivalent to a 4% reduction in life years [28]. According to the Global Burden Disease group, each 5 kg/m^2 increase in BMI was associated with a 27% increased risk of coronary heart disease (CHD) as well as an 18% increased risk for stroke [29].

Obesity is associated with increased healthcare costs. Recent estimations conclude that approximately 21% of US healthcare expenditures (total spendings of US\$ 190 billion per year) are due to treatment of obesity and obesity associated diseases. Increased costs are primarily attributed to medical expenditures such as hospitalization and drug prescriptions. In detail, obese men entail additional per-capita health care costs of 1,152 US\$ and obese women burden additional per-capita health expenditures of 3,613 US\$ per year as compared to their normal weight counterparts, respectively [30]. 20% of the annual budget of the National Institute of Health (some 6 billion US\$) is attributed to preventive measures. However, less than 10% is invested in human behavioural interventions independently associated with the major modifiable risk factors [31]. Increases in caloric intake, changes in the composition of diet and decreasing levels of physical activity are the most considerable contributors accounting for the large increase in obesity over the last decades [32-37], notwithstanding that other factors might affect overweight and obesity as well. For example, there was an intensive debate regarding the relative contribution of changes in energy intake versus energy expenditure [38, 39]. Another important point is that persons belonging to lower socioeconomic classes (with the highest poverty rates and lowest education levels) tend to consume a diet that is rich in energy but has a low nutrient density [40, 41], which makes obesity a even more important political issue.

1.4 Dietary and physiological risk factors for NCD

The Global Burden Disease working group suggests to counteract the following dietary risk factors with the following food components: a diet low in fruits and vegetables, milk,

calcium, fibre, PUFA, nuts, seeds, seafood and omega-3 fatty acids and whole grains. On the other hand alcohol use, a diet high in sodium, red meat, processed meat, sugar-sweetened beverages, trans fatty acids (TFA) and physical inactivity collectively accounted for 10% of global disability-adjusted life years in 2010 [28]. In 2010, the leading global single physiological risk factor was high blood pressure, predominantly causing CVD. Worldwide, nearly 40% of people older than 25 years are hypertensive and the prevalence of high blood pressure (systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg) has increased from 600 million to a billion in 2008 [42]. The most prominent risk factors for CVD were high BMI, high FG and high total cholesterol (TC) [28]. Biomarkers with strong predictive potential include high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerols (TG) and c-reactive protein (CRP) [43]. Flow mediated dilatation (FMD) and interleukin-6 (IL-6) are also independent risk factors for T2DM and CVD [44, 45]. Cardiorespiratory fitness (expressed as maximum oxygen uptake (VO_2 max) or metabolic equivalents) is defined as “*the ability of the circulatory, respiratory and muscular systems to supply oxygen during sustained physical activity*” and can be measured by a cycle ergometer or treadmill [46]. Strong evidence suggest that VO_2 max is associated with a reduced all-cause and cardiovascular mortality as well as cancer incidence in men and women [46-48].

1.5 Prevention of NCD by physical exercise and dietary measures

Diet and exercise are cornerstones in the prevention and therapy of overweight and obesity and associated diseases. Thereby reductions of body weight (primary fat mass

(FM) - visceral adipose tissue) are the main objectives. Energy expenditure increases with physical activity, especially with aerobic exercise training (AET) or CT (combined aerobic and resistance training) [49] and increases in resting metabolic rate could be achieved by long-term exercise intervention, especially resistance training (RT) [50, 51]. AET is known to increase the sympathetic tone and the subsequent release of adrenergic transmitters leads to an increased lipolysis especially in abdominal fat [52]. Supposedly, RT triggers the preservation and build-up of body protein thereby altering the relationship between lean body mass (LBM) and FM [53]. Several trials have shown that a combination of AET + RT induces synergistic effects on cardiovascular risk factors in T2DM patients [54-56].

The Finnish Diabetes Prevention study was one of the first long-term intervention trials showing that lifestyle intervention (hypo-caloric diet and increased physical activity) reduces the risk of T2DM compared to both a control and a pharmacological (metformin) group in individuals with abnormal glucose metabolism [57]. The “*Prevención con Dieta Mediterránea trial*”, one of the largest dietary intervention studies to date, showed that an MED rich in olive oil or nuts reduced the risk of CVD by 30% when compared to the low fat (LF) counterparts [58]. Adherence to four healthy lifestyle factors (BMI: <30 kg/m²; ≥3.5 hour/week physical activity; high intake of fruits/vegetables, whole grain bread and low meat consumption; not smoking) was associated with a 78% lower risk for developing a chronic disease (CVD, T2DM and cancer) in the EPIC-Potsdam cohort compared to non-adhering participants [3].

In contrast, the Look AHEAD trial including 5,145 overweight/obese patients with T2DM could not find any significant reduction of cardiovascular events after a median

follow-up of 9.6 years, although several cardiovascular risk factors could be improved in the intervention group [59]. In addition, the Women's Health Initiative dietary modification including 48,835 postmenopausal women, did not observe significant effects of a LF diet on BW, cardiovascular and cancer risk as well as blood lipids in an 8-year follow-up when compared to the control group [60-62].

1.6 Nutritional and physical activity guidelines in the prevention of NCD

1.6.1 Dietary Guidelines for Americans/Physical Activity Guidelines for Americans

The Dietary Guidelines for Americans 2010 (DGA) aimed to simultaneously promote health and to prevent disease in the general American population [63]. Key recommendations include the long-term maintenance of calorie balance and a healthy weight. Another major point is to focus on a diet with nutrient-dense foods and beverages. A desirable diet limits intake of sodium (<2,300 mg/d), solid fats, added sugars and refined grains. The intake of SFA should be lower than 10% of total energy content (TEC), saturated fatty acids (SFA) should be replaced by monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), cholesterol intake should be <300 mg/d and TFA intake should be as low as possible. Alcohol consumption should not exceed one drink per day (equal to 18g alcohol) for women and two drinks per day for men. Recommended foods are: vegetables (dark-green, red and orange), fruits, whole grains (at half of all grains), fat-free or low-fat milk and milk products (milk, yogurt, cheese or fortified soy beverages), seafood, lean meats and poultry, eggs, beans and peas, nuts (unsalted) and seeds. In addition, the USDA recommends two eating patterns, the USDA

Food pattern or its vegetarian adaptation as well as the Dietary approaches to stop Hypertension (DASH score) (Table 1).

Pattern	Usual U.S. intake Adults	DASH	USDA Food pattern
Food groups			
Vegetables: total	1.6 cup	2.1 cup	2.5 cup (250g)
Dark-green	0.1 cup	n.d	0.2 cup
Beans and peas	0.1 cup	see protein foods	0.2 cup
Red and orange	0.4 cup	n.d	0.8 cup
Other	0.5 cup	n.d	0.6 cup
Starchy	0.5 cup	n.d	0.7 cup
Fruit and juices	1.0 cup	2.5 cup	2.0 cup (200g)
Grains: total	180g	210g	170g
Whole grains	17g	110g	≥85g
Milk and milk products	1.5 cup	2.6 cup	3 cup
Protein foods			
Meat	70g	40g	50g
Poultry	35g	50g	40g
Eggs	10g	n.d	10g
Fish/seafood	15g	40g	35g
Beans and peas	see vegetables	10g	see vegetables
Nuts, seeds and soy products	15g	25g	17g
Oils	18g	25g	27g
Solid fats	43g	n.d	16g
Added sugars	79g	n.d	32g
Alcohol	9.9g	n.d	n.d

Table 1: Eating Pattern Comparison: Usual U.S Intake, DASH and USDA Patterns; Average Daily Intake at or adjusted to a 2,000 Calorie Level (modified according to [63])

1 cup = 225 ml; 1 cup vegetables = 125g; 1 cup fruits = 100g; n.d. = no data;

The Physical Activity Guidelines for Americans (published in 2008) focuses on recommendations for adults to achieve substantial health benefits. Statements include to exercise an equivalent of 150 minutes per week of moderate-intensity (500 metabolic equivalents minutes a week) or 75 minutes per week of vigorous-intensity aerobic activity. However, the panel also stresses the fact that people would achieve additional and more extensive health benefits (lower risk of breast/colon cancer and prevention of unhealthy weight gain) if they increase their aerobic physical activity to 300 minutes per week of moderate-intensity, or 150 minutes per week of vigorous-intensity aerobic physical activity [64] (Table 2). Additionally, the governmental guideline recommends RT (muscle-strengthening activities) that involves all major muscle groups (legs, hips, back, abdomen, chest, shoulders and arms) on two or more days per week at moderate or high intensity (defined as how much weight or force is used relative to how much a person is able to lift).

Moderate intensity
• Walking briskly (≥ 4.8 km/h, but not race-walking) • Water aerobics • Bicycling slower than 16 km/h • Tennis (doubles) • Ballroom dancing • General gardening
Vigorous intensity
• Race-walking, jogging, or running • Swimming laps • Tennis (singles) • Aerobic dancing • Bicycling ≥ 16 km/h • Heavy gardening (continuous digging or hoeing, with heart rate increases) • Hiking uphill or with a heavy backpack

Table 2: Examples of different aerobic activities and intensities [64]

1.6.2 American Heart Association/American College of Cardiology

The AHA and the ACC published an updated position paper on lifestyle management to reduce cardiovascular risk in 2013 [65]. The panel systematically reviewed the published literature and focused mainly on dietary patterns and macronutrient composition. To reduce LDL-C and blood pressure, the panel recommends the consumption of a dietary pattern with a high intake of vegetables, fruits and whole grains; moderate intake of low-fat dairy products, poultry, fish, legumes, non-tropical vegetable oils and nuts; as well as limited intake of sweets, sugar sweetened beverages and red meats. The DASH dietary pattern and the USDA pattern are expressly mentioned and recommended. An advisable

dietary pattern will not exceed intake of SFA above 5-6% of TEC and will lower sodium intake ($<2,400$ mg/d) (Table 3). With respect to physical exercise, AET is recommended in order to reduce LDL-C and non-HDL-C as well as to lower blood pressure: 3-4 sessions per week, lasting on average 40 minutes per session and involving moderate-to-vigorous intensive physical activity [65].

	HEI-2010		aHEI-2010		aMED		DASH	
	<u>Criteria for minimum score</u>	<u>Criteria for maximum score</u>	<u>Criteria for minimum score</u>	<u>Criteria for maximum score</u>	<u>Criteria for minimum score</u>	<u>Criteria for maximum score</u>	<u>Criteria for minimum score</u>	<u>Criteria for maximum score</u>
Component								
Whole grains	0 g eq/ 1000 kcal	≥42	0 g eq/ 1000 kcal	≥140g and 170 g/d (men and women)	Less than median	Median or greater	Lowest quintile	Highest quintile
Total vegetables, cup eq/1000 kcal	0	≥1.1	-	-	-	-	-	-
Vegetables excluding potatoes, cup eq/d	-	-	0	≥2.5	Less than median	Median or greater	Lowest quintile	Highest quintile
Greens and beans, cup eq/1000 kcal	0	≥0.2	-	-	-	-	-	-
Total fruit	0 cup eq/ 1000 kcal	≥0.8 cup eq/1000 kcal	0 cup eq/d	≥2 cup eq/d	Less than median	Median or greater	Lowest quintile	Highest quintile
Whole fruit, cup eq/1000 kcal	0	≥0.4	-	-	-	-	-	-
Nuts and legumes, g eq/d	-	-	0	≥28	-	-	Lowest quintile	Highest quintile
Nuts	-	-	-	-	Less than median	Median or greater	-	-
Legumes	-	-	-	-	Less than median	Median or greater	-	-
Seafood and plant proteins, g eq/1000 kcal	0	≥22	-	-	-	-	-	-
Fish	-	-	-	-	Less than median	Median or greater	-	-
Total protein foods, g eq/1000 kcal	0	≥70	-	-	-	-	-	-
Low-fat dairy, cup eq/1000 kcal	0	≥1.3	-	-	-	-	Lowest quintile	Highest quintile

FA ratio	PUFA+MUFA:SFA, <1.2	PUFA+MUFA:SFA, ≥2.5	-	-	MUFA:SFA, less than median	MUFA:SFA, Median or greater	-	-
trans fat, %	-	-	≥4	≤0.5	-	-	-	-
EPA + DHA, mg/d	-	-	0	250	-	-	-	-
PUFAs, %	-	-	≤2	≥10	-	-	-	-
Alcohol	-	-	≥3.5 and ≥2.5 drinks/d (men and women)	0.5-2 and 0.5-1.5 drinks/d (men and women)	<5 or >15 and <10 or >25 g/d (men and women)	5-15 and 10-25 g/d (men and women)	-	-
Red and processed meat, g eq/d	-	-	42	0	Median or greater	Median or greater	Highest quintile	Lowest quintile
Refined grains, g eq/1000 kcal	≥120	≤50	-	-	-	-	-	-
Empty calories, % of kcal	≥50	≤19	-	-	-	-	-	-
Sugar-sweetened beverages and fruit juices, ml eq/d	-	-	-	≥225	0	-	-	-
Sugar-sweetened beverages	-	-	-	-	-	-	Highest quintile	Lowest quintile
Sodium, g/1000 kcal	≥2.0	≤1.1	Highest decile	Lowest decile	-	-	Highest quintile	Lowest quintile

Table 3: Scoring standards for each component of the HEI-2010, AHEI-2010, aMED and DASH score according to Reedy and colleagues [66]. 1 cup fruits= 100g; 1 cup vegetables= 125g;

1.6.3 American Diabetes Association/American College of Sports Medicine

The 2013 nutrition therapy recommendations for the management of adults with diabetes by the ADA stated that there is not a ‘one size fits all’ ideal percentage of calories from carbohydrate, protein and fat for all people with diabetes. The panel recommended a variety of acceptable dietary patterns for the management of diabetes (MED, vegetarian and vegan, low-fat, low-carbohydrate and DASH) (Table 3) [67]. For overweight and obese adults with T2DM, reducing energy intake to promote weight loss has the highest level of evidence (LOE). Regarding the qualitative aspect of carbohydrates, substituting LGL foods for HGL foods may modestly improve glycaemic control. The ADA and the ACSM recommended a combination of RT and AET of at least 150 min of moderate-intensity exercise per week to improve glycaemic control in T2DM individuals [68].

1.6.4 World Cancer Research Fund/American Institute for Cancer Research

The expert report by the WCRF and the AICR published in 2007 focused on 10 recommendations to reduce the risk of cancer [69]:

1. Body fatness: be as lean as possible within the normal range of BW;
2. Physical activity: be moderately physically active, equivalent to brisk walking, for at least 30 minutes every day; as fitness improves, aim for 60 minutes or more of moderate, or for 30 minutes or more if vigorous, physical activity every day;
3. Limit consumption of energy-dense foods (prefer foods of plant origin): at least 600g of non-starchy vegetables and fruits and at least 25g non-starch polysaccharide daily;

4. Limit intake of red meat, avoid processed meat: average consumption of red meat should not be more than 300g/week;
5. Limit alcohol drinks: no more than two drinks a day for men and one drink a day for women;
6. Limit consumption of salt: 2 g sodium/day;
7. Avoid mouldy cereals or pulses: Grains and legumes;
8. Aim to meet nutritional needs through diet alone: dietary supplements are not recommended for cancer prevention;
9. Breastfeeding;
10. For cancer survivors: follow the recommendations for cancer prevention

1.7 Critical discussion of dietary guidelines

In an Open Letter to the Secretaries of the USDA as well as the US Department of Health and Human Services, published a creation of the 2015 DGA, whereby Hite and Schoenfeld criticized that the DGA are based on weak, limited and inconclusive scientific data since its first publication in 1980 culminating in the statement that the DGA have contributed to the increase of chronic disease in the US rather than preventing it [70]. The major points of criticism include the following recommendations:

- a) dietary SFA increases the risk of heart disease, although

two recent meta-analysis concluded that there is no strong scientific support for dietary recommendations that restrict SFA [71, 72];
- b) dietary cholesterol increases the risk of heart disease, although

due to a lack of evidence, nearly all other western nations have dropped their limits on dietary cholesterol [73];

c) PUFA vegetable oils reduce the risk of heart disease and should be consumed as the primary source of dietary fat, although

recent research showed that diets high in omega-6 fatty acids (linoleic acid) present in vegetable oils may actually increase risk of chronic disease or death [74].

The precarious handling of dietary guidelines and recommendations has been proposed from different sources; e.g. scientists claim to revisit current dietary recommendation for SFA as long as controversies remain [75-77]. For several years, a low-fat diet was propagated by international authorities [78, 79], however, this was adapted some years later [80]. A review by Walker and Parker published in 2014 summarized the low-fat recommendations articulated over the past 40 years and concluded that reductions in total carbohydrate intake, increases in protein intake and adoption of a Mediterranean diet (MED) seem to be more effective in inducing weight loss than reducing total fat intake [81].

1.8 Adherence to dietary guidelines and risk of NCD/cardiovascular risk factors

Correlating the adherence to dietary guidelines with indicators of chronic diseases and cardiovascular outcome parameters provided inconsistent results. On the one hand, adherence to the AHA diet and lifestyle recommendations (version 2006) was associated with a significantly lower prevalence of the metabolic syndrome in Japanese male

workers [82]. In addition, the 2006 AHA lifestyle guidelines were associated with beneficial effects on plasma HDL-C, serum insulin, CRP concentrations and 10 year risk of CHD in older Puerto Ricans [83]. Concordance with the WCRF/AICR guidelines was associated with a reduced risk of overall cancer, colorectal cancer, breast, stomach, kidney and aerodigestive cancers in the EPIC study including 386,355 participants [84]. On the other hand, adherence to the WCRF/AICR guidelines was not associated with obesity-related cancer in the Framingham offspring cohort [85]. Moreover, data from the CARDIA study suggest that the adherence to the 2005 DGA was not associated with reduced risk of T2DM and has no beneficial effects on long-term weight maintenance in American young adults [86, 87].

1.9 Systematic reviews and Meta-analyses in evidence based medicine

During the last decades, the number of systematic reviews has remarkably increased and they continue to replace narrative reviews previously been used to combine data from multiple studies. Narrative reviews are often characterized by a lack of transparency and are therefore subjectively inherent [88]. With the tremendous increase of scientific publications [89], the methodology of narrative reviews has become less useful and systematic approaches have become the preferred option. Systematic reviews are described as comprehensive and objective summaries of all relevant high quality research evidence addressing precise questions [90]. In all fields of health sciences including nutritional sciences, systematic reviews have become an important tool for the evaluation of intervention trials and the transfer of the results into evidence-based science/medicine. The use of systematic reviews and meta-analyses for lifestyle-related topics is also

becoming increasingly popular due to the accumulation of scientific data in course of the last years [91].

The term “*Evidence based medicine*” was first published in the context of medical education by Guyatt and colleagues in 1992 [92]. In 1996, Sackett and colleagues provided a more detailed definition. Randomized controlled trials (RCTs) and systematic reviews were considered to be the “*gold standard*”, although some medical questions were assumed to not require RCTs or to pressing to wait for the trials to be conducted [93].

In order to execute systematic reviews and meta-analyses in a standardized fashion, the Cochrane Collaboration (an independent organization that conduct following the principles of evidence based medicine [94]) has published an official document that provides a detailed description of the evidence synthesis methodology, i.e. the Cochrane Handbook [90]. The main goal of the evidence synthesis methodology is the reduction of bias to provide more reliable findings [95, 96]. The Cochrane Handbook defined five key characteristics for systematic reviews [90]:

1. a clearly stated set of objectives with pre-defined eligibility criteria for studies
2. an explicit, reproducible methodology
3. a systematic search that attempts to identify all studies that would meet the eligibility criteria
4. an assessment of the validity of the findings of the included studies, for example through the assessment of risk of bias; and

5. a systematic presentation and synthesis, of the characteristics and findings of the included studies

Furthermore, all authors of systematic reviews and meta-analyses of RCTs are to follow the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [97, 98], while the appropriate tool for systematic reviews and meta-analyses of observational studies is the MOOSE (Meta-analysis of Observational Studies in Epidemiology) checklist [99]. Recording a detailed protocol of each systematic review is an essential part of manuscript submission now required by most peer-review journals. The PRISMA statement consists of a 27-item checklist and a four-phase flow diagram. Particular important PRISMA checklist points include an “*explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes and study design*”; indication of a review protocol; presentation of full electronic search strategy of at least one database; study selection process; data extraction process; assessment risk of bias; description of methods to handle data and combine results; reporting of evidence synthesis and additional analyses; summary of the main findings and strength of the evidence; report sources of funding [97, 98].

A key element in systematic reviews is the statistical synthesis (meta-analysis) of individual data records of each identified study [100]. Meta-analyses provide a quantitative summary (effect size) of the individual studies included within a systematic review.

1.10 Pairwise & Network Meta-analyses

Traditional pairwise meta-analyses are used to synthesize the results of different trials evaluating the same intervention(s) to obtain an overall estimate of the treatment effect of one intervention relative to the control/intervention. A number of methodological papers can be found in the literature describing the specifics of pairwise meta-analyses (e.g. methods for combination of results from two-by-two tables [101] or the introduction of random effects meta-analysis [102]). Methodological and advances in software usability have further increased the popularity of meta-analytical techniques (i.e. quantifying heterogeneity in meta-analysis [103]). Instructional and methodological developments in the field of systematic reviews were reviews by Sutton and Higgins in 2008 [104].

In comparison to pairwise variants, network meta-analyses provide a highly useful tool to analyse data from more than two available treatments and their interactions in determining disease and health outcome parameters. When results of individual trials are pooled and if more than two treatments are available for the same disease and outcome, it is possible to develop a network of RCTs (i.e. Diet + Exercise vs. Diet, Diet + Exercise vs. Exercise, Diet vs. Exercise in the present thesis) [105] (Figure 1).

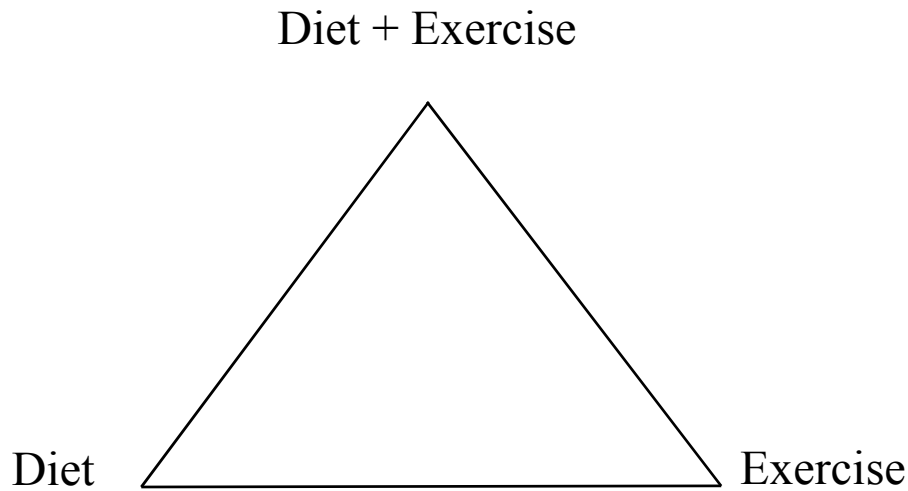


Figure 1: Network of treatments compared. A line connecting two treatments means that they have been compared in a trial.

Although particularly used in medical decision-making, network meta-analyses in research synthesis represents an interesting and helpful tool in other areas of life sciences as well [105-108]. They can be interpreted as extensions of the standard pairwise meta-analysis model that enables a simultaneous comparison of multiple interventions while preserving the internal randomisation of individual trials. Network meta-analyses have the advantage of adequately accounting for the correlation in relative effect estimates from multiple-arm trials as well as providing a single coherent summary of all the evidence [109]. Whereas the methodological theories underlying traditional pairwise meta-analyses of head-to-head comparisons are well established and accepted, network meta-analysis for both direct and indirect comparisons seems to be more complex. Therefore, probability statements should be interpreted with caution, since differences between various interventions may be small and of low clinical relevance [110, 111].

1.11 Meta-analysis in Public Health Nutrition

Evidence based dietary guidelines must consider many factors and should be based on the completeness of the available evidence [112]. Some of the discrepancies discussed above might be clarified with the help of systematic reviews and meta-analyses and there is indeed an enormous increase in the number of meta-analyses with nutrition-related topics. Although systematic reviews and meta-analyses may help to merge and ultimately enhance evidence based nutritional guidelines, some concerns were expressed regarding the validity and application to nutrition and nutritional sciences [113, 114].

RCTs in nutrition research are often prone to inherent methodological constraints. They sometimes cannot be controlled with “*true*” placebos, but rather by a limitation of certain aspects of nutrient compositions, food groups or dietary patterns. Other limitations include the lack of double blinding, poor compliance and adherence, crossover bias and drop-out. Well-designed prospective cohort studies provide important evidence with complementary strength and limitations as well. Strictly adhering to the most rigid rules of systematic reviews might result in the fact that they cannot be conducted at all in nutritional research due to these and other limitations. However, the key task of a systematic review is to provide the most comprehensive qualitative and quantitative synthesis of evidence by reducing bias and random error through its transparent, explicit, reproducible, comprehensive and rigorous processes that examine all of the available evidence [115, 116]. This cannot be achieved by relegating it to a rigid methodology locked into one type of approach for one type of study design. This, in turn calls for a new perspective on thinking about the use of meta-analyses in the context of the best and most relevant discipline-specific data [117]. Moreover, well-conducted systematic

reviews should report information of the strength and nature of all the evidence in terms of the quality (or lack thereof) of research studies, the consistency of the effect and the evidence of causality (particularly useful when there is controversy or conflicting results across the studies) [115, 118].

Although there are some limitations to incorporate systematic reviews into nutrition research, there are strong reasons to apply them as evidence based approaches for dietary guidelines. First, scientific findings about “*what one should eat*” is almost always echoed by media coverage and therefore potentially models public opinion about nutrition. Media coverage makes use of a reductionist and sentimental approach to bring their story to the readers, which may sometimes obscure actual findings of the study. Second, the field of nutrition has gone through major paradigm changes regarding dietary guidelines in the past decades [61, 119-121]. As such, recommendations for food groups, for micro- and macronutrient and dietary supplementation intake have changed exceptionally fast. In combination with a reductionist in media coverage, this may lead to extreme positions in the public opinion regarding the discipline of nutritional sciences.

Sometimes the existing data resulting from systematic reviews and meta-analyses is too limited, too conflicting, or too weak in quality and therefore insufficient evidence may be reported. A well-established tool in developing clinical or practice guidelines is assessing the quality and strength of the evidence in systematic reviews introduced by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group [122, 123]. The GRADE group used the following definition for the quality of a body of evidence: “*as the extent to which one can be confident that an estimate of effect*

or association is close to the quantity of specific interest.” The following criteria were assessed:

- risk of bias
- directness of evidence
- heterogeneity
- precision of effect estimates
- risk of publication bias

The highest quality rating is for randomized trial evidence. When appropriate, the quality of evidence should be downgraded for randomized trial evidence to moderate, low, or even very low quality evidence. In addition to the GRADE approach, the Scottish Intercollegiate Guideline Network offers a second well established tool to evaluate the quality of evidence [124]. According to the updated Scottish Intercollegiate Guideline Network handbook, the LOE were as follows:

- 1⁺⁺ high quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias
- 1⁺ well conducted meta-analyses, systematic reviews, or RCTs with a low risk of bias
- 1⁻ meta-analyses, systematic reviews, or RCTs with a high risk of bias
- 2⁺⁺ high quality systematic reviews of case control or cohort studies; High quality case control or cohort studies with a very low risk of confounding or bias and a high probability that the relationship is causal

- 2⁺ well conducted case control or cohort studies with a low risk of confounding or bias and a moderate probability that the relationship is causal
- 2⁻ well conducted case control or cohort studies with a low risk of confounding or bias and a moderate probability that the relationship is causal
- 3 well conducted case control or cohort studies with a low risk of confounding or bias and a moderate probability that the relationship is causal
- 4 well conducted case control or cohort studies with a low risk of confounding or bias and a moderate probability that the relationship is causal

2. Aims of the Thesis

The aims of the present dissertation will be splitted into four major topics (Figure 2). The nutrition part (from macronutrients- to dietary pattern), physical activity and lifestyle, i.e. a combination of both nutrition and physical exercise. The rationale of the individual aims of each meta-analyses are described in the corresponding papers (Introduction section)

2.1 Macronutrients

2.1.1 Lipids

The aim of the lipids subsection- high fat (HF) vs. LF was to conduct systematic reviews and meta-analyses to examine the long-term effects (≥ 12 months) of HF vs. LF diet consumption on

- a) blood lipids in overweight and obese participants and
- b) glycaemic control as well as cardiovascular risk factors in pre-diabetic and diabetic individuals.

The aim of the lipids subsection-dietary fatty acids was to investigate the effects of reduced and/or modified fat diets and dietary fatty acids on all-cause mortality, cardiovascular mortality and cardiovascular events in participants with established CHD.

The aim of the lipids subsection-MUFA was to conduct a systematic review and meta-analysis of cohort studies on MUFA and CVD (combining CHD and stroke), cardiovascular mortality, all-cause mortality and to distinguish between the different dietary sources of MUFA (e.g. olive oil) as well as to conduct a systematic review of

RCTs and cohort studies investigating the effects of MUFA on cardiovascular risk factors, cardiovascular events and cardiovascular death.

2.1.2 Carbohydrates

The aim of the carbohydrates subsection- LC vs. LF was to conduct a meta-analysis to compare the effects of low carbohydrate (LC) and LF regimens on flow-mediated dilatation.

The aim of the carbohydrates subsection-LGI/LGL vs. HGI/HGL was to investigate the long-term (≥ 6 months) effects of LGI/LGL as a dietary means in the management of overweight and obesity with special emphasis on its potential usability in the primary prevention of obesity-associated disorders.

2.1.3 Proteins

The aim of the proteins section was to perform a meta-analysis that compares

- a) the long-term effects of high protein (HP) vs. low protein (LP) regimens on biomarkers of obesity, cardiovascular complications as well as adverse effects of HP and
- b) the effects of HP versus LP/NP diets on parameters of renal function in adult subjects.

2.2 Dietary patterns

2.2.1 Mediterranean dietary pattern

The aim of the MED pattern was to summarize the available data regarding

- a) the impact of MED pattern on parameters of inflammation (CRP, IL-6, adiponectin, tumor-necrosis-factor-alpha (TNF- α)) and endothelial function (intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1(VCAM-1), E-Selectin, FMD),
- b) the effect of adherence to an MED pattern on the risk of T2DM and
- c) the effects of adherence to MED pattern on overall cancer risk and risk of different cancer types (e.g., colorectal, breast, gastric, prostate, aero-digestive and pancreas).

2.2.2 Diet quality indices

The aim of the diet quality indices section was to conduct a systematic review and meta-analysis of cohort studies that investigated the association of diet quality as assessed by the Healthy Eating Index (HEI), alternate Healthy Eating Index (AHEI) and DASH score and health status (i.e. risk of all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, T2DM and neurodegenerative diseases).

2.3 Physical activity

The aim of the physical activity section was to conduct systematic reviews and meta-analyses of RCTs to assess the efficacy of AET, RT and CT (AET + RT) on

- a) anthropometric outcomes, blood lipids and cardiorespiratory fitness in overweight/obese subjects and
- b) anthropometric outcomes, blood lipids, glycaemic control and cardiorespiratory fitness in T2DM patients.

2.4 Lifestyle

The aim of the lifestyle section was to conduct a systematic review with pairwise and network meta-analysis of RCTs to combine the direct and indirect evidence on the efficacy of different lifestyle long term weight-reducing interventions (diet + exercise vs. diet vs. exercise) on anthropometric parameters, blood lipids, blood pressure and cardiorespiratory fitness in participants with a BMI ≥ 25 kg/m².

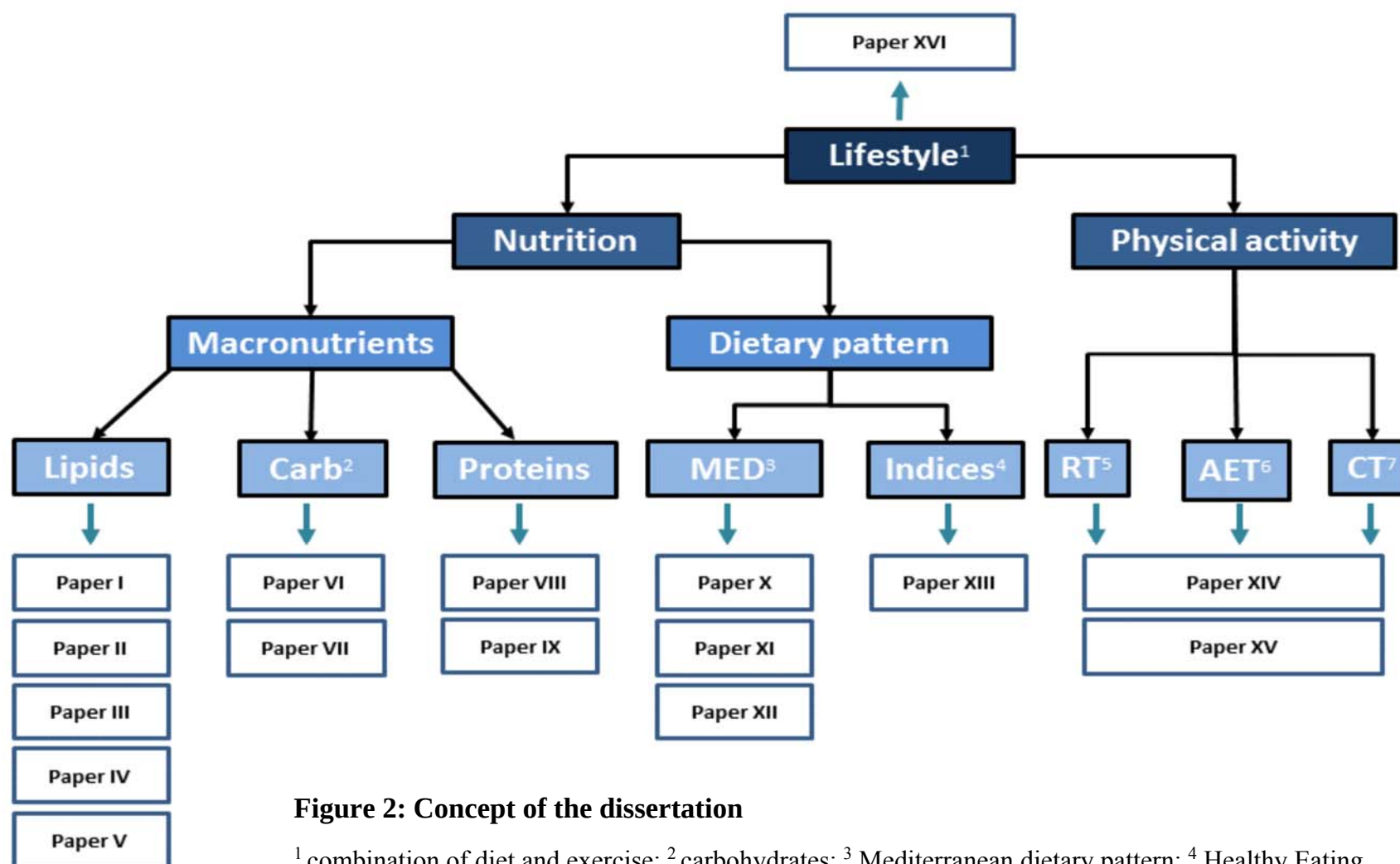


Figure 2: Concept of the dissertation

¹ combination of diet and exercise; ² carbohydrates; ³ Mediterranean dietary pattern; ⁴ Healthy Eating Index, alternate Healthy Eating Index, and Dietary approaches to stop Hypertension; ⁵ resistance training; ⁶ aerobic exercise training; ⁷ combined training (AET+RT);

3. Results

The results of the present thesis are summarised in form of the abstract of each corresponding publication (with the exception of Paper VI, which is a Letter to the Editor of the British Journal of Nutrition and is included in full text). All manuscripts mentioned in the Results section have been accepted for publication.

3.1 Macronutrients and cardiovascular risk factors/risk of CVD

3.1.1 Paper I

Comparison of effects of long-term low-fat vs high-fat diets on blood lipid levels in overweight or obese patients: a systematic review and meta-analysis

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *J Acad Nutr Diet* 2013; 113 (12): 1640-1661 (published)

Abstract

Background: Dietary fat plays an important role in the primary prevention of cardiovascular disease, but long-term (≥ 12 months) effects of different percentages of fat in the diet on blood lipid levels remain to be established. Our systematic review and meta-analysis focused on RCTs assessing the long-term effects of low-fat diets compared with diets with high amounts of fat on blood lipid levels. **Methods:** Relevant RCTs were identified searching Medline, EMBASE and the Cochrane Trial Register until March 2013. **Results:** Thirty-two studies were included in the meta-analysis. Decreases in TC (MD -4.55 mg/dl [-0.12 mmol/l], 95% CI -8.03 to -1.07; $P=0.01$) and LDL-C (MD -3.11

mg/dl [-0.08 mmol/l], 95% CI -4.51 to -1.71; $P < 0.0001$) were significantly more pronounced following low-fat diets, whereas rise in HDL-C (MD 2.35 mg/dl [0.06 mmol/l], 95% CI 1.29 to 3.42; $P < 0.0001$) and reduction in triglyceride levels (MD -8.38 mg/dl [-0.095 mmol/l], 95% CI -13.50 to -3.25; $P = 0.001$) were more distinct in the high-fat diet groups. Including only hypocaloric diets, the effects of low-fat vs high-fat diets on TC and LDL-C levels were abolished. Meta-regression revealed that lower TC was associated with lower intakes of SFA and higher intakes of PUFA and increases in HDL-C levels were related to higher amounts of total fat largely derived from MUFA (of either plant or animal origin) in HF diets (composition of which was ~17% of TEC in the form of MUFA, ~8% of TEC in the form of PUFA), whereas increases in triglyceride levels were associated with higher intakes of carbohydrates. In addition, lower LDL-C level was marginally associated with lower SFA intake. **Conclusion:** The results of our meta-analysis do not allow for an unequivocal recommendation of either low-fat or high-fat diets in the primary prevention of CVD.

3.1.2 Paper II

Comparison of the long-term effects of high-fat v. low-fat diet consumption on cardiometabolic risk factors in subjects with abnormal glucose metabolism: a systematic review and meta-analysis

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *Br J Nutr* 2014; 111 (12): 2047-2058 (published)

Abstract

Background: The aim of the present systematic review and meta-analysis was to examine the long-term effects (≥ 12 months) of HF vs. LF diet consumption on the indicators of glycaemic control as well as cardiovascular risk factors in pre-diabetic and diabetic individuals. **Methods:** Literature search was carried out using the electronic databases Medline, EMBASE and the Cochrane Trial Register until November 2013. Study-specific weighted MD were pooled using a random-effects model of the Cochrane software package Review Manager 5.1 and Stata 12.0 was used for meta-regressions. **Results:** A total of fourteen trials met the inclusion criteria and a maximum of 1,753 subjects were included in the meta-analysis. HF regimens were found to result in a significant decrease in TG levels (MD -0.19 mmol/l, 95% CI -0.23, -0.14, $P < 0.001$; $I^2 = 0\%$, $P = 0.58$) and DBP (MD -1.30 mmHg, 95% CI -1.73, -0.87, $P < 0.001$; $I^2 = 0\%$, $P = 0.60$) and a significant increase in HDL-C levels (MD 0.05 mmol/l, 95% CI 0.01, 0.08, $P = 0.01$; $I^2 = 57\%$, $P = 0.01$). In addition, MD in the reductions of FG (-0.41 mmol/l, 95% CI -0.74, -0.08, $P = 0.01$; $I^2 = 56\%$, $P = 0.02$) were significantly high in patients with T2DM adhering to a HF diet. **Conclusion:** HF and LF diets might not be of equal value in the

management of either pre-diabetes or T2DM, leading to emphasis being placed on the recommendations of HF diets.

3.1.3 Paper III

Dietary fatty acids in the secondary prevention of coronary heart disease: a systematic review, meta-analysis and meta-regression

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *BMJ Open* 2014; 4:e004487 (published)

Abstract

Background: Previous systematic reviews were not restricted to either primary or secondary prevention trials, this study aimed to investigate the effects of reduced and/or modified fat diets and dietary fatty acids on all-cause mortality, cardiovascular mortality and cardiovascular events in participants with established CHD. **Methods:** Systematic review, meta-analysis and univariate/multivariate meta-regression. Electronic searches for RCTs comparing reduced/modified fat diets versus control diets were performed in MEDLINE, EMBASE and the Cochrane Library. Pooled effects were calculated using an inverse-variance random effect meta-analysis. Random effects univariate and multivariate meta-regressions were performed including changes in all types of dietary fatty acids. **Results:** Overall, 12 studies enrolling 7,150 participants were included in the present systematic review. No significant risk reduction could be observed considering all-cause mortality (risk ratio (RR) 0.92, $P=0.60$; $I^2=59\%$) and cardiovascular mortality (RR 0.96, $P=0.84$; $I^2=69\%$), combined cardiovascular events (RR 0.85, $p=0.30$; $I^2=75\%$) and myocardial infarction (RR 0.76, $P=0.13$; $I^2=55\%$) comparing modified fat diets versus control diets. This results could be confirmed for the reduced fat versus control diets (RR 0.79, $P=0.47$; $I^2=0\%$), (RR 0.93, $P=0.66$; $I^2=0\%$), (RR 0.93, $P=0.71$;

$I^2=57\%$) and (RR 1.18, $P=0.26$; $I^2=18\%$). The multivariate and univariate model showed no significant associations between the independent variables and the changes from SFA, MUFA, PUFA and linoleic acid. Sensitivity analyses did not reveal a significant risk reduction for any outcome parameter when PUFA was increased in exchange for SFA.

Conclusions The present systematic review provides no evidence (moderate quality evidence) for the beneficial effects of reduced/modified fat diets in the secondary prevention of CHD. Recommending higher intakes of PUFA in replacement of SFA was not associated with risk reduction.

3.1.4 Paper IV

Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *Lipids in Health and Disease* 2014; 13:154 (published)

Abstract

Background: The aim of the present meta-analysis of cohort studies was to focus on MUFA and CVD, cardiovascular mortality as well as all-cause mortality and to distinguish between the different dietary sources of MUFA. **Methods:** Literature search was performed using the electronic databases PubMed and EMBASE until June 2nd, 2014. Study specific RR and hazard ratios were pooled using a inverse variance random effect model. **Results:** Thirty-two cohort studies (42 reports) including 841,211 subjects met the objectives and were included. The comparison of the top versus bottom third of the distribution of a combination of MUFA (of both plant and animal origin), olive oil, oleic acid and MUFA:SFA ratio in each study resulted in a significant risk reduction for: all-cause mortality (RR 0.89, 95% CI 0.83, 0.96, P= 0.001; I²= 64%), cardiovascular mortality (RR 0.88, 95% CI 0.80, 0.96, P= 0.004; I²= 50%), cardiovascular events (RR 0.91, 95% CI 0.86, 0.96, P= 0.001; I²= 58%) and stroke (RR 0.83, 95% CI 0.71, 0.97, P= 0.02; I²= 70%). Following subgroup analyses, significant associations could only be found between higher intakes of olive oil and reduced risk of all-cause mortality, cardiovascular events and stroke, respectively. The MUFA subgroup analyses did not reveal any significant risk reduction. **Conclusion:** The results indicate an overall risk

reduction of all-cause mortality (11%), cardiovascular mortality (12%), cardiovascular events (9%) and stroke (17%) when comparing the top versus bottom third of MUFA, olive oil, oleic acid and MUFA:SFA ratio. MUFA of mixed animal and vegetable sources per se did not yield any significant effects on these outcome parameters. However, only olive oil seems to be associated with reduced risk. Further research is necessary to evaluate specific sources of MUFA (i.e. plant vs. animal) and cardiovascular risk.

3.1.5 Paper V

Monounsaturated Fatty Acids and Risk of Cardiovascular Disease: Synopsis of the Evidence Available from Systematic Reviews and Meta-Analyses

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *Nutrients* 2012; 4 (12): 1989-2007 (published)

Abstract

Background: No dietary recommendations for MUFA are given by the National Institute of Medicine, the USDA, European Food and Safety Authority and the ADA. In contrast, the Academy of Nutrition and Dietetics and the Canadian Dietetic Association both promote <25% MUFA of daily total energy consumption, while the American Heart Association sets a limit of 20% MUFA in their respective guidelines. The present review summarizes systematic reviews and meta-analyses of RCTs and cohort studies investigating the effects of MUFA on cardiovascular and diabetic risk factors, cardiovascular events and cardiovascular death. **Methods:** Electronic database Medline was searched for systematic reviews and meta-analyses using “*monounsaturated fatty acids*”, “*monounsaturated fat*” and “*dietary fat*” as search terms with no restriction to calendar date or language. Reference lists and clinical guidelines were searched as well. **Results:** Sixteen relevant papers were identified. Several studies indicated an increase of HDL-C and a corresponding decrease in TG following a MUFA-rich diet. The effects on total and LDL-C appeared not consistent, but no detrimental effects on blood lipids were observed. Values for SBP and DBP were found to be reduced both during short- and long-term protocols using high amounts of MUFA as compared to low-MUFA diets. In T2DM

subjects, MUFA exerted a hypoglycaemic effect and reduced glycosylated haemoglobin (HbA1c) in the long term. Data from meta-analyses exploring evidence from long-term prospective cohort studies provide ambiguous results with respect to the effects of MUFA on risk of CHD. One meta-analysis reported an increase in CHD events, however, most meta-analyses observed a lesser number of cases in participants subjected to a high-MUFA protocol. **Conclusion:** Although no detrimental side effects of MUFA-rich diets were reported in the literature, there still is no unanimous rationale for MUFA recommendations in a therapeutic regimen. Additional long-term intervention studies are required to characterized efficacy and effectiveness of recommending MUFA-rich diet among general and clinical populations.

3.1.6 Paper VI

Low-carbohydrate diets impair flow-mediated dilatation: evidence from a systematic review and meta-analysis

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *Br J Nutr* 2013; 110 (5): 969-970 (*published*)

Letter to the Editor, no abstract available



Letter to the Editor

Low-carbohydrate diets impair flow-mediated dilatation: evidence from a systematic review and meta-analysis

With great interest, we read the meta-analysis of Bueno *et al.*⁽¹⁾ 'Very-low-carbohydrate ketogenic diet *v.* low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials', published ahead of print in the *British Journal of Nutrition*⁽¹⁾. In their article, the authors compared the effects of very-low-carbohydrate (VLC) diets *v.* low-fat (LF) diets on the biomarkers of obesity and their associated disorders. With respect to different outcomes between both the regimens, VLC diets turned out to be more effective in lowering body weight, TAG levels and diastolic blood pressure and in increasing HDL-cholesterol levels. However, one established risk factor of CVD, i.e. LDL-cholesterol, still turned out to be harmfully affected by the VLC regimen, most probably attributable to the larger amounts of saturated fat in the diet⁽¹⁾. In their discussion, the authors stated that future meta-analyses should investigate the impact of low carbohydrates (LC) *v.* LF on other important pathological markers, e.g. endothelial function, in order to further assess the safety of LC dietary therapies. This is reasonable, since evidence from prospective cohort studies has shown that endothelial dysfunction represents an independent risk factor for the development of many CVD including atherosclerosis⁽²⁾. We, therefore, carried out a meta-analysis to compare the effects of LC and LF regimens on flow-mediated dilatation (FMD). FMD of the brachial artery is a non-invasive measure of endothelial function, furthermore reflecting the local bioavailability of endothelium-derived vasodilators, especially NO. Inflammation of the endothelium is regarded to play a major role in the destabilisation of atherosclerotic lesions, therefore paving the way for future CVD events⁽²⁾.

A literature search was performed using the electronic databases MEDLINE (until April 2013), EMBASE (until April 2013) and the Cochrane Trial Register (until April 2013), with restrictions to randomised controlled trials, but no restriction to language using the following search terms: low fat diet AND endothelial function and low carbohydrate diet AND endothelial function (Supplementary material, available online). Studies were included in the meta-analysis if they met all of the following criteria: (1) randomised controlled design with a minimum intervention period of 3 weeks; (2) comparing a LC diet ($\leq 45\%$ carbohydrates of total energy content, TEC) with a LF diet ($\leq 30\%$ fat of TEC)⁽³⁾; (3) report of post-intervention values with standard deviations; when the results of a study were published more than once, only the most recent or most complete article was included in the analysis; (4) participants age > 18 years; (5) exclusion of subjects with CHD.

A meta-analysis was carried out in order to determine the pooled effect of the intervention in terms of weighted mean differences (WMD) between the post-intervention values of the LC group and those of the LF group. All data were analysed using the Review Manager 5.1 software, provided by the Cochrane Collaboration (<http://ims.cochrane.org/revman>). Overall, six trials with a sample size of 210 subjects were included⁽⁴⁻⁹⁾. Study duration ranged between 3.5 weeks and 12 months. Decreases in FMD (WMD: -1.01 (95% CI $-1.83, -0.19$), $P=0.02$) were significantly more pronounced following consumption of LC diets than following that of LF diets (Fig. 1). The I^2 test showed very low heterogeneity ($I^2 = 10\%$).

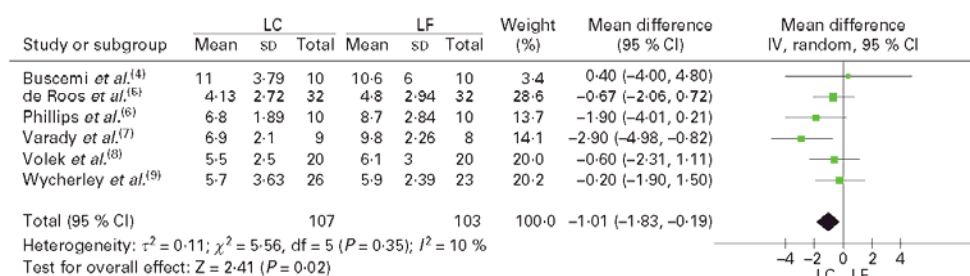


Fig. 1. Forest plot showing pooled weighted mean differences (WMD) with 95% CI for flow-mediated dilatation (%) of six randomised controlled low-carbohydrate diet studies. For each low-carbohydrate trial, ■ represents the point estimate of the intervention effects. The horizontal line joins the lower and upper limits of the 95% CI of these effects. The area of ■ reflects the relative weight of the study in the respective meta-analysis. At the bottom of the graph, ◆ represents the pooled WMD with 95% CI.



In our meta-analysis, LC dietary protocols were associated with a significant decrease in FMD when compared with their LF counterparts. A recent meta-analysis of observational studies including a sample size of 5·547 subjects has observed that a 1% decrease in FMD is associated with a 13% increase in the risk of future cardiovascular events⁽²⁾. In another recent meta-analysis of cohort studies carried out by Noto *et al.*⁽¹⁰⁾, an association between LC diets and increased risk of all-cause mortality could be observed, although not for cardiovascular (CVD) mortality as well as CVD incidence⁽¹⁰⁾. It should be noted that in direct comparison with the meta-analysis carried out by Bueno *et al.*⁽¹⁾, we had to modify the inclusion criteria, since only a few dietary intervention trials had reported FMD as an outcome parameter. However, in consideration of the fact that LC was associated with a higher all-cause mortality risk, further trials are required to confirm the mechanisms of FMD impairment following LC regimens.

Supplementary material

To view the supplementary material for this article, please visit <http://dx.doi.org/10.1017/S000711451300216X>

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3.1.7 Paper VII

Long-term effects of low glycaemic index/load vs. high glycaemic index/load diets on parameters of obesity and obesity-associated risks: A systematic review and meta-analysis

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *Nutr Metab Cardiovasc Dis* 2013; 23 (8): 699-706 (published)

Abstract

Background: The aim of the present meta-analysis was to investigate the long-term effects of glycaemic index-related diets in the management of obesity with a special emphasis on the potential benefits of LGI/LGL in the prevention of obesity-associated risks. **Methods:** Electronic searches for RCTs comparing low glycaemic index/load (LGI/LGL) versus high glycaemic index/load (HGI/HGL) diets were performed in Medline, EMBASE and the Cochrane Library. Outcome of interest markers included anthropometric data as well as biomarkers of CVD and glycaemic control. Study specific weighted mean differences (MD) were pooled using a random effect model. **Results:** 14 studies were included in the primary meta-analysis. Weighted MD in change of CRP [MD -0.43 mg/dl, (95% CI -0.78 to -0.09), $P=0.01$] and fasting insulin [MD -5.16 pmol/L, (95% CI -8.45 to -1.88), $P=0.002$] were significantly more pronounced in benefit of low GI/GL diets. However decrease in fat free mass [MD -1.04 kg (95% CI -1.73 to -0.35), $P=0.003$] was significantly more pronounced following low GI/GL diets as well. No significant changes were observed for blood lipids, anthropometric measures, HbA1c and FG. Sensitivity analysis was performed for RCTs excluding subjects with T2DM.

Decreases in CRP and FI remained statistically significant in the low GI/GL subgroups.

Conclusions: The present systematic review provides evidence for beneficial effects of long-term interventions administering a low glycaemic index/load diet with respect to fasting insulin and pro-inflammatory markers such as CRP which might prove to be helpful in the primary prevention of obesity-associated diseases.

3.1.8 Paper VIII

Long-term effects of low-fat diets either low or high in protein on cardiovascular and metabolic risk factors: a systematic review and meta-analysis

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *Nutrition Journal* 2013, 12:48 (published)

Abstract

Background: Meta-analyses of short-term studies indicate favourable effects of higher protein vs. lower protein diets on health outcomes like adiposity or cardiovascular risk factors, but their long-term effects are unknown. **Methods:** Electronic databases (Medline, EMBASE and Cochrane Trial Register) were searched up to August 2012 with no restriction to language or calendar date. A random effect meta-analysis was performed using the Software package by the Cochrane Collaboration Review Manager 5.1. Sensitivity analysis was performed for RCTs with a Jadad Score ≥ 3 and excluding T2DM patients. **Results:** 15 RCTs met all objectives and were included in the present meta-analysis. No significant differences were observed for BW, waist circumference (WC), FM, blood lipids (i.e. TC, LDL-C, HDL-C, TG), CRP, DBP, SBP, FG and HbA1c. In contrast, improvements of fasting insulin was significantly more pronounced following HP diets as compared to the LP counterparts (MD -0.71 μ IU/ml, 95% CI -1.36 to -0.05, $P=0.03$). Sensitivity analysis of high quality RCTs confirmed the data of the primary analyses, while exclusion of studies with diabetic subjects resulted in an additional benefit of high-protein diets with respect to a more marked increase in HDL-C. **Conclusion:** According to the present meta-analysis of long-term RCTs, high-protein diets exerted

neither specific beneficial nor detrimental effects on outcome markers of obesity, cardiovascular disease or glycaemic control. Thus, it seems premature to recommend high-protein diets in the management of overweight and obesity.

3.1.9 Paper IX

Comparison of High vs. Normal/Low Protein Diets on Renal Function in Subjects without Chronic Kidney Disease: A Systematic Review and Meta-Analysis

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *PLoS ONE* 2014; 9(5): e97656 (published)

Abstract

Background: It was the aim of the present systematic review and meta-analysis to investigate the effects of HP versus LP/NP diets on parameters of renal function in subjects without chronic kidney disease. **Methods:** Queries of literature were performed using the electronic databases Medline, EMBASE and the Cochrane Trial Register until 27th February 2014. Study specific weighted MD were pooled using a random effect model by the Cochrane software package Review Manager 5.1. **Results:** 30 studies including 2,160 subjects met the objectives and were included in the meta-analyses. HP regimens resulted in a significantly more pronounced increase in glomerular filtration rate (GFR) [MD 7.18 ml/min/1.73 m², 95% CI 4.45 to 9.91, P< 0.001], serum urea [MD 1.75 mmol/l, 95% CI 1.13 to 2.37, P< 0.001] and urinary calcium excretion [MD 25.43 mg/24h, 95% CI 13.62 to 37.24, P< 0.001] when compared to the respective LP/NP protocol. **Conclusion:** HP diets were associated with increased GFR, serum urea, urinary calcium excretion and serum concentrations of uric acid. In the light of the high risk of kidney disease among obese, weight reduction programs recommending HP diets especially from animal sources should be handled with caution.

3.2 Dietary patterns and cardiovascular risk factors/risk of chronic disease

3.2.1 Paper X

Mediterranean dietary pattern, inflammation and endothelial function: a systematic review and meta-analysis of intervention trials

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *Nutr Metab Cardiovasc Dis* 2014; 24 (9): 929-939 (published)

Abstract

Background: High adherence to a MED is associated with reduced all-cause and cardiovascular mortality risk. To our knowledge, there is no systematic review and meta-analysis of RCTs that has compared the effects of an MD on outcomes of endothelial function and inflammation. **Methods:** Literature search was performed using the electronic databases Medline, EMBASE and the Cochrane Trial Register. Inclusion criteria were: RCTs, 19+ years of age and minimum intervention period of 12 weeks. Study specific weighted MD were pooled using a random effect model. **Results:** Seventeen trials including 2,300 subjects met the objectives. MED regimens resulted in a significantly more pronounced increase in flow mediated dilatation [MD 1.86 %, 95% CI 0.23 to 3.48, $P=0.02$; $I^2=43\%$] and adiponectin [MD 1.69 $\mu\text{g/ml}$, 95% CI 0.27 to 3.11, $P=0.02$; $I^2=78\%$], while CRP [MD -0.98 mg/l, 95% CI -1.48 to -0.49, $P<0.0001$; $I^2=91\%$], IL-6 [MD -0.42 pg/ml, 95% CI -0.73 to -0.11, $P=0.008$; $I^2=81\%$] and intracellular adhesion molecule-1 [MD -23.73 ng/ml, 95% CI -41.24 to -6.22 $P=0.008$; $I^2=34\%$] turned out to be significantly more decreased. **Conclusion:** The results of the present

meta-analysis provide evidence that an MED decreases inflammation and improves endothelial function.

3.2.2 Paper XI

Adherence to a Mediterranean diet and risk of diabetes: a systematic review and meta-analysis

Authors: Lukas Schwingshackl, Benjamin Missbach, Jürgen König, Georg Hoffmann

Journal: *Public Health Nutr* 2014; (*Epub ahead of print*) (*published online: 22.08.2014*)

Abstract

Background: Adherence to a MED is associated with significant improvements in health status. However, to date no systematic review and meta-analysis has summarized the effects of MED adherence on the risk of T2DM. **Methods:** Electronic searches for RCTs and cohort studies were performed in Medline, SCOPUS, EMBASE and the Cochrane Trial Register until 2 April 2014. Pooled effects were calculated by an inverse-variance random-effect meta-analysis using the statistical software Review Manager 5.2 by the Cochrane Collaboration. **Results:** One RCT and eight prospective cohort studies (122,810 subjects) published between 2007 and 2014 were included for meta-analysis. For highest v. lowest adherence to the MED score, the pooled RR was 0.81 (95% CI 0.73, 0.90, $P < 0.0001$, $I^2 = 55\%$). Sensitivity analysis including only long-term studies confirmed the results of the primary analysis (pooled RR= 0.75; 95% CI 0.68, 0.83, $P < 0.00001$, $I^2 = 0\%$). The Egger regression test provided no evidence of substantial publication bias ($P = 0.254$). **Conclusions:** Greater adherence to a MED is associated with a significant reduction in the risk of diabetes (19%; moderate quality evidence). These results seem to be clinically relevant for public health, in particular for encouraging a Mediterranean-like dietary pattern for primary prevention of T2DM.

3.2.3 Paper XII

Adherence to Mediterranean diet and risk of cancer: A systematic review and meta-analysis of observational studies

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *Int J Cancer* 2014; 135 (8): 1884-1897 (published)

Abstract

Background: The aim of this research study was to meta-analyse the effects of adherence to MED on overall cancer risk and different cancer types. **Methods:** Literature search was performed using the electronic databases Medline, SCOPUS and EMBASE until January 10, 2014. Inclusion criteria were cohort or case-control studies. Study specific RRs were pooled using a random effect model by the Cochrane software package Review Manager 5.2. **Results:** Twenty-one cohort studies including 1,368,736 subjects and 12 case-control studies with 62,725 subjects met the objectives and were enclosed for meta-analyses. The highest adherence to MED category resulted in a significantly risk reduction for overall cancer mortality/incidence (cohort; RR 0.90, 95% CI 0.86 to 0.95, $P < 0.0001$; $I^2 = 55\%$), colorectal (cohort/case-control; RR 0.86, 95% CI 0.80 to 0.93, $P < 0.0001$; $I^2 = 62\%$), prostate (cohort/case-control; RR 0.96, 95% CI 0.92 to 0.99, $P = 0.03$; $I^2 = 0\%$) and aero-digestive cancer (cohort/case-control; RR 0.44, 95% CI 0.26 to 0.77, $P = 0.003$; $I^2 = 83\%$). Non-significant changes could be observed for breast cancer, gastric cancer and pancreatic cancer. The Egger regression tests provided limited evidence of substantial publication bias. **Conclusion:** High adherence to a MED is

associated with a significant reduction in the risk of overall cancer mortality (10%), colorectal cancer (14%), prostate cancer (4%) and aerodigestive cancer (56%).

3.2.4 Paper XIII

Diet quality as assessed by the Healthy Eating Index, the Alternate Healthy Eating Index and the DASH score and health outcomes: a systematic review and meta-analysis of cohort studies

Authors: Lukas Schwingshackl, Georg Hoffmann

Journal: *J Acad Nutr Diet* 2015 (accepted 09.12.2014)

Abstract

Background: Dietary patterns consider synergistic effects compared to isolated foods or nutrients on health outcomes. The aim of this systematic review and meta-analysis was to examine the associations of diet quality as assessed by the HEI, the AHEI and the DASH score and the risk of all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, T2DM and neurodegenerative diseases. **Methods:** A literature search was performed using the electronic databases Medline, SCOPUS and EMBASE with an end date of May 10, 2014. Study specific RR were pooled using a random effect model by the Cochrane software package Review Manager 5.2. **Results:** Fifteen cohort studies (34 reports) including 1,020,642 subjects, met the criteria and were included in the meta-analysis. Diets of the highest quality, as assessed by the HEI, AHEI and DASH score, resulted in a significant risk reduction for all-cause mortality [RR 0.78, 95% CI 0.76 to 0.80, $P < 0.00001$; $I^2 = 61\%$, 95% CI 20% to 81%], CVD (incidence or mortality) [RR 0.78, 95% CI 0.75 to 0.81, $P < 0.00001$; $I^2 = 45\%$, 95% CI 13% to 66%], cancer (incidence or mortality) [RR 0.85, 95% CI 0.82 to 0.88, $P < 0.00001$; $I^2 = 77\%$, 95% CI 68% to 84%] and T2DM [RR 0.78, 95% CI 0.72 to 0.85, $P < 0.00001$; $I^2 = 74\%$,

95% CI 52% to 86%]. Differences observed for neurodegenerative diseases were non-significant. The Egger regression tests provided no evidence of publication bias.

Conclusion: Diets that score highly on the HEI, AHEI and DASH are associated with a significant reduction in the risk of all-cause mortality, CVD and cancer and T2DM by 22%, 22%, 15% and 22%, respectively and therefore is of high public health relevance.

3.3 Physical activity and cardiovascular risk factors

3.3.1 Paper XIV

Impact of Different Training Modalities on Anthropometric and Metabolic Characteristics in Overweight/Obese Subjects: A Systematic Review and Network Meta-Analysis

Authors: Lukas Schwingshackl, Sofia Dias, Barbara Strasser, Georg Hoffmann

Journal: *PLoS ONE* 2014; 8(12): e82853 (published)

Abstract

Background: The aim of this systematic review of RCTs was to compare the effects of AET, RT and CT on anthropometric parameters, blood lipids and cardiorespiratory fitness in overweight and obese subjects. **Methods:** Electronic searches for RCTs were performed in Medline, EMBASE and the Cochrane Trial Register. Inclusion criteria were: BMI: ≥ 25 kg/m², 19+ years of age, supervised exercise training and a minimum intervention period of 8 weeks. Anthropometric outcomes, blood lipids and cardiorespiratory fitness parameters were included. Pooled effects were calculated by inverse-variance random effect pairwise meta-analyses and Bayesian random effects network meta-analyses. **Results:** 15 trials enrolling 741 participants were included in the meta-analysis. Compared to RT, AET resulted in a significantly more pronounced reduction of BW [MD -1.15 kg, P= 0.04], WC [MD -1.10 cm, P= 0.004] and FM [MD -1.15 kg, P= 0.001] respectively. RT was more effective than AET in improving LBM [MD 1.26 kg, P< 0.00001]. When comparing CT with RT, MD in change of BW [MD -

2.03 kg, $P < 0.0001$], WC [MD -1.57 cm, $P = 0.0002$] and FM [MD -1.88 kg, $P < 0.00001$] were all in favour of CT. Results from the network meta-analyses confirmed these findings. **Conclusion:** Evidence from both pairwise and network meta-analyses suggests that CT is the most efficacious means to reduce anthropometric outcomes and should be recommended in the prevention and treatment of overweight and obesity whenever possible.

3.3.2 Paper XV

Impact of different training modalities on glycaemic control and blood lipids in patients with type 2 diabetes: a systematic review and network meta-analysis

Authors: Lukas Schwingshackl, Benjamin Missbach, Sofia Dias, Jürgen König, Georg Hoffmann

Journal: *Diabetologia* 2014; 57(9): 1789-1797 (published)

Abstract

Background: This study aimed to systematically review RCTs comparing the effects of AET, RT and CT on glycaemic control and blood lipids in patients with T2DM. **Methods:** This study aimed to systematically review RCTs comparing the effects of AET, RT and CT on glycaemic control and blood lipids in patients with T2DM. **Results:** A total of 14 trials enrolling 915 participants were included. AET was more effective than RT in improving HbA1c levels (MD -0.20% [-2.2 mmol/mol]; 95% CI -0.32, -0.08; P= 0.0007, 10 trials/515 participants) and FG (-0.9 mmol/l; 95% CI -1.71, -0.09; P= 0.03, 8 trials/245 participants). Compared with AET, CT resulted in a significantly more pronounced reduction in HbA1c (MD -0.17% [-1.87 mmol/mol]; 95% CI -0.31, -0.03; P= 0.02, 9 trials/493 participants). Compared with RT, the MD of the change in HbA1c (MD -0.62%, [-6.82 mmol/mol]; 95% CI -0.95, -0.30; P= 0.0002, 5 trials/362 participants), FG (MD -1.99 mmol/l; 95% CI -3.07, -0.90; P= 0.0003, 3 trials/ 99 participants) and TG (MD -0.28 mmol/l; 95% CI -0.46, -0.10; P= 0.003, 4 trials/213 participants) were all in favour of CT. The exclusion of trials with a high risk of bias yielded only non-significant results. **Conclusion:** The present data suggest that CT might be the most efficacious exercise

modality to improve glycaemic control and blood lipids. Interpretation with respect to clinical relevance is limited by the low quality of the studies included and the limited information on the clinically important outcomes or adverse effects of exercise.

3.4 Lifestyle intervention and cardiovascular risk factors

3.4.1 Paper XVI

Impact of long-term lifestyle programmes on weight loss and cardiovascular risk factors in overweight/obese participants: a systematic review and network meta-analysis

Authors: Lukas Schwingshackl, Sofia Dias, Georg Hoffmann

Journal: *Systematic Reviews* 2014; 3:130 (published)

Abstract

Background: The aim of this meta-analysis was to compare the long-term efficacy of diet plus exercise vs. diet, diet + exercise vs. exercise and diet vs. exercise on anthropometric outcomes and cardiovascular risk factors in overweight and obese participants. **Methods:** Electronic searches were performed in Medline and the Cochrane Central Register of controlled trials. Inclusion criteria were as follows: BMI ≥ 25 kg/m² and a minimum intervention period including follow-up of ≥ 12 months. Outcomes of interest were as follows: anthropometric parameters, blood lipids, blood pressure and cardiorespiratory fitness. Pooled effects were calculated using pairwise random effects and Bayesian random effects network meta-analysis. Results of the corresponding fixed effects models were compared in sensitivity analyses. **Results:** Overall, 22 trials (24 reports) met the inclusion criteria and 21 (including 3,521 participants) of them were included in the quantitative analysis. As compared with diet, diet + exercise resulted in a significantly more pronounced reduction in BW [MD -1.38 kg, 95% confidence interval (CI) -1.98 to -0.79] and FM (MD -1.65 kg, 95% CI -2.81 to -0.49], respectively. When

comparing diet + exercise with exercise, MD in change of BW (-4.13 kg, 95% CI -5.62 to -2.64), WC (-3.00 cm, 95% CI -5.81 to -0.20) and FM (-3.60 kg, 95% CI -6.15 to -1.05) was in favour of combined diet and exercise, respectively. Comparing exercise vs. diet, diet resulted in a significantly more pronounced decrease in BW (MD -2.93 kg, 95% CI -4.18 to -1.68) and FM (MD -2.20 kg, 95% CI -3.75 to -0.66). Diet + Exercise yielded also the greatest reductions with respect to blood lipids and blood pressure when compared to single applications of diet and exercise, respectively. Results from the network meta-analyses confirmed these findings. **Conclusions:** Moderate-quality evidence from the present network meta-analysis suggests that diet + exercise can be highly recommended for long-term obesity management. Furthermore, the evidence suggests a moderate superiority of diet over exercise with respect to anthropometric outcomes.

4. Discussion

The Discussion section will give a short summary of the main results of every single meta-analysis of the four major topics (Macronutrients, Dietary patterns, Physical activity and Lifestyle) and will compare the present findings with other meta-analyses and current lifestyle (diet and physical activity) guidelines for the prevention of chronic disease.

4.1 Macronutrients

The first nutrition subsection summarizes the effects of different macronutrient compositions (with respect to lipids, carbohydrates and proteins) on cardiovascular risk factors and hard clinical endpoints in primary and secondary prevention of CVD. For ease of orientation, the corresponding papers (and reference) are given in parenthesis.

4.1.1 Lipids

LF vs. HF in overweight/obese individuals (Paper I, [125])

In overweight and obese individuals, a LF diet was associated with a significant decrease in LDL-C, TC and HDL-C and a significant increase in TG as compared to the higher fat counterpart. The meta-regression analysis showed that increases in TC levels were associated with higher SFA and lower PUFA contents of HF regimens, whereas a rise in HDL-C level was related to higher amounts of total fat with an emphasis on MUFA content. These results seem to be controversial, because the ideal dietary regimen for reducing the risk of CVD would be one that lowers LDL-C level and raises HDL-C level simultaneously [125]. The evidence that increased levels of LDL-C cause CVD is

impressive [126, 127]. However, the role of decreased HDL-C as an isolated risk factor is still discussed controversially [43, 128]. In a recent meta-analysis, comparison of regimens providing higher amounts of fat with LF diets yielded improvements in HDL-C and triglyceride levels, but at the same time increases in LDL-C and TC levels were seen following the HF regimens as well [129]. Other systematic reviews of RCTs comparing LC vs LF diets reported inconsistent effects on TC and HDL-C levels, significant improvements in triglyceride levels, but detrimental effects on LDL-C levels [130, 131].

LF vs. HF in patients with abnormal glucose metabolism (Paper II, [132])

In individuals with abnormal glucose metabolism, the primary analysis revealed a favourable effect of HF diet consumption with respect to TG levels, DBP and HDL-C levels. By contrast, an advantage of an LF protocol could be observed when compared with its usual/HF counterpart with respect to FI values in the subgroup analyses. A significant decrease in FG values was found in patients with T2DM subjected to an HF dietary regimen [132]. Kodama et al. [133] observed significant increases in TG levels as well as decreases in HDL-C levels in patients with T2DM subjected to an LF diet than in those of patients subjected to an HF protocol focusing on short-term intervention trials. Weighing moderate-fat diets against a regimen with LF intake, Cao et al. [134] found significant changes in TG levels, HDL-C levels and TC:HDL-C ratio in favour of the moderate-fat protocol. Following a meta-regression analysis, the authors concluded that at least the changes observed in HDL-C levels were correlated with total fat as well as unsaturated fat intake, suggesting that a reduction in carbohydrate intake with

simultaneous increase in unsaturated fat (MUFA and PUFA) intake would exert a beneficial effect on plasma lipoproteins.

Dietary fatty acids in the secondary prevention of CVD (Paper III, [135])

In this systematic review of 7150 participants with established CHD or coronary artery disease comparing reduced and/or modified fat diets versus control diets, no significant risk reduction (moderate quality of evidence) for all-cause mortality, cardiovascular mortality, cardiovascular events and myocardial infarction could be observed. Sensitivity analysis indicates that it seems reasonable to modify this general recommendation by promoting higher dietary n-3 PUFA as a substitute for SFA [135]. Small but nevertheless significant reductions in cardiovascular events could be observed by Hooper et al. [136] as well as Truswell [137] following their respective meta-analyses (sensitivity analysis) of intervention studies investigating the effects of a modification of fatty acid intake as a secondary preventive measure in patients with CVD. A meta-analysis of RTCs by Mozaffarian and colleagues [138] reported a significant reduction in CHD events (by 19%) following the replacement of SFA by PUFA. However, adapting (for secondary prevention) and updating (including new data from the Sydney Diet Heart Study) this meta-analysis [138] as was carried out in a sensitivity analysis investigating the replacement of SFA by PUFA in the present study, resulted in neither beneficial nor detrimental effects on all outcome parameters [135].

MUFA, olive oil and health status (Paper IV, [139])

Comparison of the top versus the bottom third of combined MUFA subgroups (MUFA, olive oil, oleic acid and MUFA: SFA) was associated with reduced risk of all-cause mortality (11%), cardiovascular mortality (12%), combined cardiovascular events (9%) and stroke (17%). In the ensuing subgroup analyses, this significant correlation could only be observed between higher intakes of olive oil and reduced risk of all-cause mortality, cardiovascular events and stroke, respectively. In contrast, MUFA of mixed animal and plant origin did not result in any significant effects with respect to these outcome parameters. Thus, it seems possible that olive oil represents the crucial factor of influence for the protective health effects observed in the primary analysis [139]. A meta-analysis of case-control, cohort and intervention studies showed that olive oil was associated with a reduced risk of CHD in case-control studies and reduced risk of stroke in cohort studies [140]. Furthermore, a meta-analysis of 19 observational studies indicates that olive oil intake is inversely related to cancer prevalence [141].

MUFA umbrella review (Paper V, [142])

In the present umbrella review, only meta-analyses (n=16) were included, which indicates a high LOE, i.e., from 2+ to 1+++ according to the Scottish Intercollegiate Guidelines Network indicate levels of evidence [124]. Apart from the fact that several meta-analyses and meta-regressions observed benefits of MUFA on cardiovascular risk factors, it should be noted that most meta-analyses did not report significant negative effects of a MUFA-rich diet on CVD risk factors. With respect to the favourable influences of MUFA found in studies recruiting healthy volunteers or patients with diabetes and CHD respectively,

some reservations still remain. Due to various in-homogeneities, the results of different studies are far from being conclusive [142].

4.1.2 Carbohydrates

LC vs LF and FMD (Paper VI, [143])

LC dietary protocols were associated with a significant decrease in FMD when compared with their LF counterparts [143]. A recent meta-analysis of observational studies including a sample size of 5,547 subjects has observed that a 1% decrease in FMD is associated with a 13% increase in the risk of future cardiovascular events [45]. In another recent meta-analysis of cohort studies carried out by Noto et al. [144], an association between low carbohydrate diets and increased risk of all-cause mortality could be observed, although not for CVD mortality as well as CVD incidence.

LGI/LGL vs. HGI/HGL (Paper VII, [145])

In summary, decreases in CRP, FI and fat free mass were significantly more pronounced in the LGI/LGL diet groups as compared to their HGI/HGL counterparts, while all other parameters under investigation were affected in a comparable fashion [145]. Systematic reviews of cohort studies suggested that HGI/HGL diets are associated with an increased risk for the development and manifestation of T2DM and CVD [146-149]. A systematic review of observational and intervention studies showed, that 5 out of 9 observational studies reported lower concentrations of CRP or IL-6 among persons with a LGI/LGL [150].

4.1.3 Proteins

HP vs. LP and cardiovascular risk factors (Paper VIII, [151])

The meta-analysis was restricted on HP as well as LP diets providing $\leq 30\%$ of TEC in the form of fat to prevent potential bias due to variations in total fat intake. None of the dietary protocols turned out to be superior to its counterpart with regard to the biomarkers under investigation. Following primary analysis, decreases in FI were significantly more pronounced in HP diets. However, this was no longer valid after inclusion of high quality trials only in the secondary analysis [151]. Taken together, these results are in discrepancy with a recent meta-analysis by Santesso and co-workers [152] who reported weight loss, WC, HDL-C, TG, SBP, DBP and FI to be significantly more improved following short- and long-term HP diets as compared to LP protocols. The different findings might at least in part be explained by the fact that only long-term studies with a duration ≥ 12 months were included in the present meta-analysis. A recent meta-analysis of long-term intervention trials (≥ 12 months) comparing higher protein, low carbohydrate diets resulted in significant decrease in weight, fat loss, TG and FI [153].

HP vs. LP renal function (Paper IX, [154])

The main findings suggest that subjects following an HP diet presented themselves with increased GFR, serum urea and urinary calcium excretion, respectively. Further increases in serum concentrations of uric acid could be observed in those individuals following an HP regimen, when the trial by Jenkins et al. [155] was excluded from the analysis due to the fact, that it was the only study using vegetable protein exclusively as a supplement

[154]. The potential detrimental effects of HP diets on kidney function are still discussed controversially. In a long-term study in rats, feeding an HP diet (35% of TEC) resulted in a significant reduction in BW, however this was accompanied by 17% higher kidney weights, a 3-fold raise in proteinuria, larger glomeruli and a 27% increase in creatinine clearance as compared to the NP (15% of TEC) feed rats, respectively [156]. A recent review of the evidence concluded that HP diets with a daily protein content of more than 25% of energy or more than 2 to 3 g/kg BW is not advisable for the general population [157].

4.1.4 Dietary recommendations for macronutrients

The acceptable macronutrient distribution for adults set by The Food and Nutrition Board of the Institute of Medicine [158] range between 45-65% for carbohydrates, 10-35% for protein and 20-35% for fat (with a special emphasis on limiting SFA and TFA). The ADA suggests that there is no ideal percentage of calories from carbohydrate, protein and fat for all people with diabetes [67].

Fatty acids

SFA: The current AHA/ACC guidelines promote to reduce the total fat intake to less than 35% of TEC and SFA intake to less than 7% of TEC, respectively in the primary and secondary prevention of CVD [159]. The DGA and the Food and Agriculture Organization/WHO recommended <10% of TEC in the form of SFA and the WHO stated that SFA should be replaced with PUFAs [63, 160].

MUFA: No dietary recommendations for MUFA are given by the Institute of Medicine, the USDA, the European Food and Safety Authority and the ADA. In contrast, the Academy of Nutrition and Dietetics as well as the Canadian Dietetic Association both promote <20% of daily total energy consumption in the form of MUFA, while the AHA sets a limit of 20% MUFA in their respective guidelines [142, 161, 162].

PUFA: The US Dietary Reference intake [158] and the AHA [159] both recommended 5-10% of TEC in the form of PUFA, the Academy of Nutrition and Dietetics gives a corresponding range of 3-10% [161], whereas no specific recommendations is given by the DGA [63].

Carbohydrate quality: The ADA suggests that substituting low-glycaemic load foods for higher-glycaemic load foods may modestly improve glycaemic control [67].

4.2 Dietary patterns

In recent years, dietary pattern analysis has been used increasingly as an alternative method to traditional single nutrient analysis [163]. The use of dietary patterns avoids focusing on single foods or nutrients that can be correlated with, or interact with each other and assesses combinations of food that alone may have effects too small to be identified. Therefore, analysis of food consumption patterns has become a common tool for studying the association between diet and health [164, 165].

4.2.1 Mediterranean dietary pattern

MED inflammation, endothelial function (Paper X, [166])

The present systematic review and meta-analysis showed that a MED pattern significantly improved markers of inflammation such as CRP, IL-6 and AD as well as parameters of endothelial function measured as FMD and ICAM-1 [166]. Regarding the validity of these outcome parameters, consistent evidence indicates that CRP is an independent risk factor for CVD and CHD [167]. IL-6 is generally accepted as an acute phase inflammatory reactive protein produced upon stimulation by TNF- α and IL-1 [168] and a known trigger of CRP synthesis [169, 170]. A recent meta-analysis suggested a 13% increase in the risk of future CVD events for every 1% decrease in FMD [45]. By transferring these data to the findings of the present meta-analysis, reduction in CVD risk following an MED will be approximately 24% stronger as compared to the respective control groups. A meta-analysis of intervention trials comparing MED pattern to LF diets showed a more favourable effects on BW, BMI, SBP and DBP, FG, TC and CRP [171].

MED risk of T2DM (Paper XI, [172])

The meta-analysis including >100,000 subjects revealed a significant association between the highest vs. lowest adherence to MED pattern and decreased risk of T2DM (19% risk reduction). The overall quality of evidence rated according to the GRADE guidelines for the main outcome was moderate [172]. Since cut-off and MED scores varied between studies, a sensitivity analysis was performed investigating the association between a 2-point increase of adherence to the MED score (defined by Trichopoulou [173]) and the

risk of T2DM. A 2-point increase in MED score was associated with a 7% risk reduction in T2DM. A very recent meta-analysis of intervention trials provides evidence that a MED pattern improves outcomes of glycaemic control, BW and cardiovascular risk factors in T2DM patients [174]. This finding is in line with the presented meta-analysis and provides further mechanistic evidence for the preventive effects of a MED pattern for T2DM.

MED risk of cancer (Paper XII, [175])

The main findings of this meta-analysis suggest that the highest adherence to MED category was associated with a significant reduction of overall risk of cancer mortality/incidence by 10%. The risk of colorectal cancer could be reduced by 14% and the risk of prostate cancer by 4%. No significant risk reduction could be observed for breast, gastric and pancreatic cancer. However, a subgroup analysis pooling case-control studies showed that the risk of breast cancer could be reduced by 18% when adhering to an MED. The association between adherence to MED and risk of aero-digestive cancer provides evidence for risk reduction from case-control studies, which could not be confirmed by pooling the cohort studies regarded to provide a higher LOE [175]. The 2nd update of a comprehensive meta-analysis of Sofi and colleagues showed that 2-point increase in MED score was associated with a 4% reduction in neoplastic disease [176-178].

4.2.2 Diet quality indices (Paper XIII, [179])

The main findings of this meta-analysis suggest that diets of the highest quality, as assessed by the HEI, AHEI and DASH score, were associated with a significant reduction of all-cause mortality risk by 22%, risk of CVD by 22%, risk of cancer by 15% and risk of T2DM by 22%, respectively [179]. In accordance with a recent meta-analysis it was shown that a DASH like diet significantly reduces the risk of CVD by 20%, of CHD by 21%, of stroke by 19% and of heart failure by 29% [180]. Furthermore, a meta-analysis of RCTs indicate that the DASH diet was associated with improvements in insulin sensitivity [181].

4.2.3 Dietary pattern recommendations

The AHA and the ACC recommended the following equivalent dietary patterns to reduce cardiovascular risk: DASH dietary pattern, the USDA food pattern, or the AHA diet [65]. The DGA included the USDA food pattern, the DASH dietary pattern and the MED pattern in their eating pattern recommendations [63]. In their most recent position paper, the ADA reviewed the following eating patterns: MED, vegetarian and vegan, low-fat, low-carbohydrate and DASH. In people with T2DM, a Mediterranean-style, MUFA-rich eating pattern may benefit glycaemic control and CVD risk factors and can therefore be recommended as an effective alternative to a lower-fat, higher-carbohydrate eating pattern. The panel concluded that there is no “*ideal*” conclusive eating pattern that is expected to benefit all individuals with diabetes [67].

4.3 Physical activity

There is a plethora of evidence suggesting that regular physical activity is one of the most important components of a healthy lifestyle, linking physical activity to a broad cluster of health benefits [182].

AET, RT, CT in overweight and obese individuals (Paper XIV, [183])

The main findings of this meta-analysis suggest that in subjects with a BMI ≥ 25 kg/m², AET is more efficient in reducing BW, WC and FM as well as in increasing VO₂ max uptake when compared to RT, respectively. However, RT turned out to be more suitable when it comes to an improvement of LBM. Furthermore, the present results provide evidence that a combined intervention seems to be the most promising tool for management of overweight and obesity. CT was more powerful in reducing anthropometric risk factors like BW, WC or FM when compared to RT and more effective in raising LBM when compared to AET [183]. Several meta-analyses investigated the independent effects of AET on anthropometric and cardio-metabolic risk factors, providing evidence for reductions in BMI, BW, WC, visceral adipose tissue and increasing HDL-C and VO₂ max uptake [184-188]. With respect to RT, some meta-analyses reported reductions of SBP, DBP and CRP [189-191].

AET, RT, CT in type 2 diabetes patients (Paper XV, [192])

The results of the present meta-analyses showed that, in patients with established T2DM, AET might be more effective in reducing HbA1c and FG when compared with RT. CT was more powerful in reducing HbA1c compared with AET and more effective in

reducing HbA1c, FG and TG when compared with RT. However, these results could not be confirmed when only low risk of bias studies were included. Pooling both direct and indirect evidence on AET, RT and CT via network meta-analysis demonstrated that CT was the most efficacious exercise intervention regarding its impact on HbA1c, FG, HDL, TG, DBP and BW (with the respective probabilities of being ranked best following Bayesian network meta-analysis of 94%, 94%, 78%, 99%, 84% and 97%) [192]. A recent meta-analysis comparing RT with AET concluded that there is no evidence that RT differs from AET in its impact on cardiovascular risk factors and safety [193]. A dose-response meta-regression analysis by Umpierre et al. [194] summarised the effects of CT, AET and RT on glycaemic control in patients with T2DM and concluded that the reduction in HbA1c was associated with exercise frequency in supervised AET and with the weekly volume of RT in supervised CT. The importance of supervised training has been demonstrated by Umpierre and colleagues, who showed that structured training compared with training advice only significantly improved glycaemic control in patients with T2DM [195]. Regarding the optimal dose, the authors speculated that there should be a minimal amount of AET (33 min per session) to elicit the effects of high-volume RT in CT [194]. The results of this meta-analysis are in line with data published by Chudyk and colleagues [196] comparing CT, AET and RT with control groups. The authors concluded that RT is not significantly related to changes in HbA1c levels or to changes in SBP in patients with T2DM if it is not combined with other forms of exercise [197]. This paper is the first network meta-analysis comparing the pooled effects of AET, RT and CT on glycaemic control, blood pressure and blood lipids in patients with T2DM and add

important scientific contribution to clarify which exercise modality offers the most benefits in the management of T2DM.

4.3.1 Physical activity recommendations

The 2007-ACSM/AHA physical activity guidelines for healthy adults (18 to 65 years of age) recommended moderate-intensity aerobic physical activity for a minimum of 30 min on five days each week or vigorous-intensity aerobic physical activity for a minimum of 20 min on three days each week. To increase muscular strength and endurance for a minimum of two days each week, 8-10 exercises should be performed on two or more non-consecutive days per week using the major muscle groups. To maximize strength development, a counteracting resistance (weight) should be used that allows 8-12 repetitions of each exercise resulting in volitional fatigue [198, 199]. According to the ADA and the ACSM, a combination of RT and AET of at least 150 min of moderate-intensity exercise per week should be performed to improve glycaemic control in T2DM individuals [68]. The Physical Activity Guidelines for Americans (version 2008) recommended 150 minutes per week of moderate-intensity or 75 minutes per week of vigorous-intensity aerobic activity [64].

4.4 Lifestyle

The combination of diet and exercise is the basis of lifestyle intervention in the prevention of overweight and obesity and associated diseases [200].

Diet/Exercise vs. Diet vs. Exercise and cardiovascular risk factors (Paper XVI, [201])

The primary findings of this systematic review and meta-analysis of long-term trials are that a combination of Diet + Exercise is more effective in reducing BW compared to either Diet or Exercise in overweight and obese participants [201]. Furthermore, improvements in WC, waist-to-hip ratio and FM were significantly more pronounced following Diet + Exercise compared to either Diet or Exercise. The combination of Diet + Exercise is also the most powerful lifestyle intervention to reduce blood lipids and blood pressure. Compared to Exercise, a dietary intervention resulted in significantly greater reductions in BW, FM and SBP. Pooling both direct and indirect evidence on Diet + Exercise, Diet and Exercise via network meta-analysis demonstrated that Diet + Exercise was the most efficacious exercise intervention regarding its impact on BW, WC, FM, TC, LDL-C, TG, DBP and VO₂ max [201]. The findings of this meta-analysis, i.e. diet being significantly more effective in reducing BW than exercise, are supported by results of a 2006 Cochrane review [202] and are consistent with results of another meta-analysis [203]. The amount of weight loss yielded by Diet + Exercise can be compared to the results of the most effective pharmacological interventions such as orlistat (4.12/3.1 kg at 12 months) [204, 205].

4.4.1 Lifestyle recommendations

The ACC and the AHA, the DGA, the WCRF/AICR and the ADA all recommended a combination of “*healthy*” diet and exercise to prevent chronic disease, such as CVD, cancer and T2DM [63, 65, 68, 69].

4.5 Limitations

In addition to the strengths of systematic reviews and meta-analyses (i.e. evidence-synthesis of individual data records of each study), there are several limitations, which are probably more effective for lifestyle interventions and observational studies of nutrition and exercise-related topics than for pharmacological or dietary supplement intervention trials. Meta-analyses are limited by the included study types and cohort studies are at risk of various potential sources of bias. For example, response bias as well as errors in the assessment of diets is a persistent problem when analysing associations between diet and disease. A major limitation of nutritional intervention trials is the heterogeneity of various aspects and characteristics of the study protocols. Therefore, it is not surprising that the RCTs included in the meta-analyses of this thesis varied regarding the type(s) of diets used (energy restriction, isoenergetic), definitions of diets, study population, intervention time and nutritional assessment, volume of exercise, differences in dietary intake and physical activity performed outside the monitoring and supervision by the investigators, as well as long-term follow-up periods. Moreover, all systematic reviews did not consider unpublished results (publication bias) and it cannot be excluded that these results may have had at least a moderate impact on the effect size estimates.

In 2014, Heaney postulated some guidelines for optimal design of systematic reviews and meta-analyses in the field of nutritional sciences. According to his standards, the following rules should be complied with [206]:

- “The individual studies selected for review or meta-analysis must themselves have met the following criteria (Basal nutrient status must be measured, used as an inclusion criterion for entry into study and recorded in the report of the trial; the intervention (i.e., change in nutrient exposure or intake) must be large enough to change nutrient status and must be quantified by suitable analyses; the change in nutrient status produced in those enrolled in the trials must be measured and recorded in the report of the trial; the hypothesis to be tested must be that a change in nutrient status (not just a change in diet) produces the sought-for effect; co-nutrient status must be optimized in order to ensure that the test nutrient is the only nutrition related, limiting factor in the response.”
- “All included studies must have started from the same or similar basal nutrient status values.”
- “All included studies must have used the same chemical form of the nutrient and, if foods are used as the vehicle for the test nutrient, all studies must have employed the same food matrix.”
- “All included studies must have the same co-nutrient status.”
- “All included studies must have had approximately equal periods of exposure to the altered intake.”

By strictly abiding to these rules/criteria, the number of studies suitable for systematic reviews and meta-analyses in the field of nutritional sciences would be very limited. Heaney’s guidelines lay focus on analysing single nutrient components (i.e. vitamin and mineral supplements) and their effects on health outcome parameters. For this reason,

they are most likely not applicable for the studies carried out in this thesis, e.g. meta-analysing dietary patterns.

5. Conclusion and Future Aspects

Systematic reviews and meta-analyses become increasingly popular, which is underlined by the rapid growth of scientific output: in 2010, eleven new systematic reviews were estimated to be published daily [91]. In 2012, a new journal called “*Systematic Reviews*” was launched [207] accepting systematic reviews and meta-analysis in the broad field of health sciences only (and systematic review protocols). The most recent refinement in systematic review research methods & reporting includes the PRISMA-P 2015 statement. It describes how to report item (i.e. the rationale of the systematic review and pre-planned methodological and analytic approach) for drafting systematic review and meta-analysis protocols [208, 209]. This is an essential step in the development of standardized methods, to increase the availability and accessibility of a priori methods, to minimise weakness [97, 98] and to reduce both the duplication of effort as well as the publication bias of systematic reviews [210]. Nutrition research has been criticized on numerous occasions [211]. Systematic reviews and meta-analysis can help to improve nutrition based dietary guidelines by incorporating more homogenous dietary recommendations. However, one has to keep in mind that systematic reviews are only as good as the studies they pool together. Therefore, the risk of bias should be reported in a comprehensive way for every systematic review and the results should be interpreted accordingly [212]. When synthesizing statistical evidence for lifestyle modifications, network meta-analytical approaches may become an important method regarding the various forms of dietary regimens and training protocols suggested to provide health benefits.

The present dissertation adds profound scientific knowledge for evidence-based lifestyle recommendations in the prevention of NCD. In particular, the main results of the

dissertation suggest that a combination of RT and AET should be highly recommended as the primary exercise prescription in the prevention and management of chronic disease. With respect to dietary intake, future dietary recommendation should not focus primarily on nutrients (i.e. macronutrients), but on dietary patterns and specific foods.

6. Summary

Introduction: Lifestyle is a crucial factor in the prevention of non-communicable diseases. Large prospective cohort studies have shown that 60-75% of coronary events and 36% of cancer incidents can be explained by factors such as unhealthy diets, overweight, obesity, physical inactivity, smoking and inadequate alcohol intake. In 2013, the number of deaths worldwide and throughout all age groups amounted to nearly 55 million people, with 70% of them caused by non-communicable diseases. 32% of global fatalities were caused by cardiovascular or circulatory disease, followed by cancer mortality with 15%. By means of systematic reviews and meta-analyses, the objective of the present dissertation (divided into four major topics: macronutrients, dietary pattern, physical activity and lifestyle) was to add scientific knowledge for guidelines in the field of public health aimed at the prevention of chronic diseases.

Methods: The studies presented in this thesis were planned, conducted and reported in adherence to standards (PRISMA, MOOSE, Cochrane Handbook) of quality for reporting meta-analyses. Pairwise and Network meta-analyses and meta-regression were performed for synthesis of quantitative evidence. Assessment of methodological quality was carried out using the risk of bias assessment tool by the Cochrane Collaboration for RCTs or the Newcastle Ottawa Scale for observational studies. For pairwise meta-analyses, data were analysed using the Review Manager software (version 5.1 and 5.2), for meta-regression analyses, the statistical package Stata (versions 12 and 13) was used and network meta-analyses were conducted using Markov chain Monte Carlo simulation implemented with the open-source software WinBUGS (version 1.4.3). In addition, Funnel plots to assess potential publication bias were generated for the majority of outcome parameters.

Results/Conclusion: Based upon new systematic reviews and meta-analyses, the present dissertation adds important scientific knowledge for evidence-based public health recommendations, especially nutrition related topics for the prevention of chronic diseases. In particular, the main results of the dissertation suggest that future dietary recommendations should not focus primarily on nutrients (i.e. macronutrients), but rather on dietary patterns and specific foods. With respect to physical exercise as a modifiable lifestyle factor, a combination of RT and AET should be highly recommended as the primary exercise regimen in the prevention and management of non-communicable diseases.

Zusammenfassung

Einleitung: Der Lebensstil ist einer der wichtigsten Faktoren in der Prävention von nicht übertragbaren Krankheiten. Große Kohorten-Studien haben gezeigt, dass 60-75% der koronaren Ereignisse und 36% der Krebsfälle auf Risikofaktoren wie ungesunde Ernährung, Übergewicht, Adipositas, körperliche Inaktivität, Rauchen und inadäquater Alkoholkonsum zurückgeführt werden können. Die Zahl aller Todesfälle weltweit lag im Jahr 2013 bei ca. 55 Million Menschen, ~70% dieser Sterbefälle wurden durch nicht übertragbare Krankheiten verursacht. 32% der globalen Todesfälle konnten auf Herz-Kreislauf-erkrankungen zurückgeführt werden, gefolgt von Tumorerkrankungen mit 15%. Die vorliegende Dissertation (untergliedert in vier große Abschnitte: Makronährstoffe, Ernährungsmuster, körperliche Aktivität, und Lebensstil) hatte zum Ziel, mit Hilfe von systematischen Übersichtsarbeiten und Meta-Analysen neue wissenschaftliche Erkenntnisse für Empfehlungen zur Prävention solcher Erkrankungen zu liefern.

Methoden: Die in dieser Arbeit vorgestellten Studien wurden unter Berücksichtigung gängiger Qualitätsstandards (PRISMA, MOOSE, Cochrane Handbook) zur Durchführung von Meta-Analysen geplant, durchgeführt und dokumentiert. Zur quantitativen Evidenzsynthese wurden sowohl direkte und indirekte Meta-Analysen als auch Meta-Regressionen durchgeführt. Die Bewertung der methodischen Qualität der einzelnen Studien erfolgte mit dem „risk of bias assessment tool“ der Cochrane Collaboration für randomisiert kontrollierte Studien und mit der Newcastle Ottawa Skala für Beobachtungsstudien. Für alle direkten meta-analytischen Vergleiche wurde das Programm Review Manager in den Versionen 5.1 und 5.2 verwendet, für Meta-

Regressionen kam das Statistikpaket Stata in den Versionen 12 und 13 zur Anwendung und für die indirekten Vergleiche wurde die Markov-Ketten-Monte-Carlo Simulation des open-source Programms WinBUGS 1.4.3 berechnet. Zusätzlich wurden für die meisten Parameter Funnel Plots erstellt, um einen potenziellen Publikationsbias zu identifizieren.

Resultate/Konklusion: Die folgende Dissertation liefert wichtige wissenschaftliche Erkenntnisse für evidenzbasierte Public Health Empfehlungen. Im Besonderen wurden ernährungswissenschaftliche Fragestellungen untersucht, die für die Prävention von chronischen Erkrankungen von Bedeutung sind. Die Ergebnisse dieser Dissertation zeigen, dass zukünftige Ernährungsempfehlungen nicht auf Ebene einzelner Nährstoffe gegeben werden sollten. Der Fokus sollte vielmehr auf Ernährungsmuster und spezielle Lebensmittelgruppen gelegt werden. Im Hinblick auf körperliche Aktivität als beeinflussbarem Lebensstilfaktor zeigt diese Arbeit, dass eine Kombination von Kraft- und Ausdauertraining in der Primärprävention von chronischen Erkrankungen sehr empfehlenswert ist.

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8. Curriculum vitae

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Resume

Born 1986 in Bruneck (BZ), Italy
2000-2005 Grammar School in Sports, Sterzing
2005-2008 Bachelor Degree in Dietetics (108/110), University of Applied Sciences, Bozen, Italy; Catholic University of the Sacred Heart, Rome, Italy
2005-2008 Internships: Medical University Innsbruck and Graz, Olympic base Munich
2009-2012 Master of Science in „Applied Nutritional Sciences“, University of Health Sciences and Medical Informatics, UMIT-Hall, Austria
Since July 2012: PhD student, University of Vienna, Department of Nutritional Sciences

Professional Qualifications

Languages: German (native), Italian, English
Statistical software: Review Manager, STATA, WinBUGS, SAS
Supervision and mentoring of Master and Bachelor students

Lecturer

2010-2012: Teacher at high school level: chemistry/physics, biology, anatomy/physiology

Since July 2012: University of Vienna, Department of Nutritional Sciences: Practical courses for Bachelor students in “Nutritional Sciences”;

Research topics

Research Synthesis

Evidence-based cardiovascular nutrition

Systematic reviews, meta-analysis, meta-regression, network meta-analysis

Ad hoc Reviewer

Journal of the American College of Nutrition

Obesity Reviews

European Journal of Clinical Nutrition

British Journal of Nutrition

Hormone and Metabolic Research

PLoS ONE

Endocrine

Public Health Nutrition

British Journal of Cancer

Diabetes Research Clinical Practice

BMC Public Health

European Journal of Nutrition

Diabetologia

British Medical Journal

Honors and awards

KWA-Fellowship 2014, University of Vienna

Invited Talks

1. Schwingshackl, L. (2014). Dietary fatty acids in the secondary prevention of coronary heart disease: a systematic review, meta-analysis and meta-regression. 42th Austrian Diabetes Conference, 20th -22th November; Salzburg, Austria.

Poster Presentations on scientific meetings (with abstracts)

1. Schwingshackl, L., & Hoffmann, G. (2014). Adherence to Mediterranean diet and risk of cancer: a systematic review and meta-analysis of observational studies. III World Congress of Public Health Nutrition, November 9th – 12th, Las Palmas de Gran Canaria, Spain (abstract: International Journal of Community Nutrition)
2. Schwingshackl, L., & Hoffmann, G. (2014). Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. III World Congress of Public Health Nutrition, November 9th – 12th, Las Palmas de Gran Canaria, Spain (abstract: International Journal of Community Nutrition)
3. Schwingshackl, L., Missbach, B., Dias, S., König, J., & Hoffmann, G. (2014). Impact of different training modalities on glycaemic control and blood lipids in type 2 diabetes subjects: a systematic review and network meta-analysis. Congress

of the European College of Sport Science, July 2nd – 5th, Amsterdam, Netherlands
(abstract: Book of Abstracts)

4. Hoffmann, G., & Schwingshackl, L., (2013). Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. IUNS, International Congress of Nutrition, September 15th – 20th, Granada, Spain (abstract: Annals of Nutrition & Metabolism)
5. Schwingshackl, L., & Hoffmann, G. (2013). Comparison of long-term high-fat vs. low-fat diets on glycemic control and cardiovascular risk factors in subjects with abnormal glucose metabolism: a systematic review and meta-analysis. IUNS, International Congress of Nutrition, September 15th – 20th, Granada, Spain (abstract: Annals of Nutrition & Metabolism)
6. Schwingshackl, L., Strasser, B., & Hoffmann, G. (2013). Impact of different training modalities on anthropometric and metabolic characteristics in overweight/obese subjects: a systematic review and meta-analysis. Congress of the European College of Sport Science, June 26th – 29th, Barcelona, Spain (abstract: Book of Abstracts)
7. Schwingshackl, L., & Hoffmann, G. (2013). Saturated fatty acids in the secondary prevention of coronary heart disease: a systematic review and meta-analysis. Deutscher Physiologen Kongress, March 2nd – 5th, Heidelberg, Germany (abstract: Acta Physiologica)
8. Schwingshackl, L., & Hoffmann, G. (2012). Comparison of long-term low-fat versus high-fat diets on blood lipids: a systematic review and meta-analysis.

Winter Meeting of the Nutrition Society: Dietary Strategies for the Management of Cardiovascular Risk, December 11th – 12th, London, Great Britain (abstract: Proceedings of the Nutrition Society)

9. Schwingshackl, L., & Hoffmann, G. (2012). Monounsaturated fatty acids and risk of cardiovascular disease: evidence from systematic review. Dreiländertagung Ernährung, September 27th – 28th, Vienna, Austria (abstract: Ernährung/Nutrition)
10. Schwingshackl, L., & Strasser, B. (2011). Effects of monounsaturated fatty acids on glycaemic control in patients with abnormal glucose metabolism: a systematic review and meta-analysis. ÖGE Jahrestagung, September 19th–21th, Vienna, Austria

9. Appendix

Paper I-XVI



Comparison of Effects of Long-Term Low-Fat vs High-Fat Diets on Blood Lipid Levels in Overweight or Obese Patients: A Systematic Review and Meta-Analysis

Lukas Schwingshackl, MSc; Georg Hoffmann, PhD

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ABSTRACT

Dietary fat plays an important role in the primary prevention of cardiovascular disease, but long-term (≥ 12 months) effects of different percentages of fat in the diet on blood lipid levels remain to be established. Our systematic review and meta-analysis focused on randomized controlled trials assessing the long-term effects of low-fat diets compared with diets with high amounts of fat on blood lipid levels. Relevant randomized controlled trials were identified searching MEDLINE, EMBASE, and the Cochrane Trial Register until March 2013. Thirty-two studies were included in the meta-analysis. Decreases in total cholesterol (weighted mean difference -4.55 mg/dL [-0.12 mmol/L], 95% CI -8.03 to -1.07 ; $P=0.01$) and low-density lipoprotein (LDL) cholesterol (weighted mean difference -3.11 mg/dL [-0.08 mmol/L], 95% CI -4.51 to -1.71 ; $P<0.0001$) were significantly more pronounced following low-fat diets, whereas rise in high-density lipoprotein (HDL) cholesterol (weighted mean difference 2.35 mg/dL [0.06 mmol/L], 95% CI 1.29 to 3.42 ; $P<0.0001$) and reduction in triglyceride levels (weighted mean difference -8.38 mg/dL [-0.095 mmol/L], 95% CI -13.50 to -3.25 ; $P=0.001$) were more distinct in the high-fat diet groups. Including only hypocaloric diets, the effects of low-fat vs high-fat diets on total cholesterol and LDL cholesterol levels were abolished. Meta-regression revealed that lower total cholesterol level was associated with lower intakes of saturated fat and higher intakes of polyunsaturated fat, and increases in HDL cholesterol levels were related to higher amounts of total fat largely derived from monounsaturated fat (of either plant or animal origin) in high-fat diets (composition of which was $\sim 17\%$ of total energy content in the form of monounsaturated fatty acids, $\sim 8\%$ of total energy content in the form of polyunsaturated fatty acids), whereas increases in triglyceride levels were associated with higher intakes of carbohydrates. In addition, lower LDL cholesterol level was marginally associated with lower saturated fat intake. The results of our meta-analysis do not allow for an unequivocal recommendation of either low-fat or high-fat diets in the primary prevention of cardiovascular disease.

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BETWEEN 2009 AND 2010, THE PREVALENCE OF obesity in the United States was 35.5% among men and 35.8% among women.¹ Moreover, obesity represents a worldwide epidemic with serious consequences. Data from the European Prospective Investigation into Cancer and Nutrition have shown that overall as well as abdominal adiposity increase mortality rates.² Obesity is

an established risk factor for the development of cardiovascular disease (CVD), which continues to be the most common cause of death in the Western world since 1970.^{3,4} Several prospective studies indicated the importance of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides as biomarkers and risk factors for CVD. Among others, the Multiple Risk Factor Intervention Trial⁵ has shown that total cholesterol is an evidence-based risk factor for coronary heart disease, and a meta-analysis of prospective cohort studies indicated that triglyceride level is an independent risk factor for CVD.⁶ Both total cholesterol level and LDL cholesterol level are correlated with coronary events in a linear fashion, and an exponential relationship has been reported in prospective cohort and clinical studies.^{7,8}

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Table 1. General characteristics of randomized controlled intervention trials included in the meta-analysis of studies assessing the long-term effects of low-fat (LF) diets compared with diets with high amounts of fat (HF) on blood lipid levels

Reference	Participants, BMI ^a , % diabetes	Age (y), women (%), men (%)	Duration (y)	Dietary Intervention	Dietary protocol fat (%), protein (%), carbohydrates (%) (source of carbohydrates)	Energy restricted (kcal)	Dietary assessment	Drop-out rate (%)	Study quality (Jadad score)
Anderson and colleagues, 1992 ²⁴	146 nd ^b 0	40.6 nd	1	LF ^c vs control	25, 20, 55 (↑ grains, vegetables, fruits, and legumes) >30	no no	3-d Food records	21 22	2
Brehm and colleagues, 2009 ²⁹	95 35.9 100	56.5 64 36	1	LF vs MUFA ^d	25, 15, 60 (↑ starches, fruits, vegetables) 40, 15, 45, 20 MUFA (olive and canola oils)	−200 to −300 −200 to −300	3-d Food records	16 31	2
Brinkworth and colleagues, 2009 ³⁰	75 33.5 0	51.5 64 36	1	LF vs LC ^e	30, 24, 46 (↑ grains, vegetables, fruits, and legumes) 61, 35, 4	1,430 women, 1,670 men 1,430 women, 1,670 men	Food record	31 42	2
Dale and colleagues, 2009 ³¹	200 31.85 0	45 100	2	LF vs MUFA	25-30, 15-20, 55 (↑ multigrain bread, legumes, oat cereals, basmati rice, fruit, and vegetables) 35, 25, 40, 21 MUFA (nuts, avocado, olive and canola oils)	no no	3-d Dietary intake record	10 16	4
Dansinger and colleagues, 2005 ³²	80 35 nd	49 48 52	1	LF vs LC	10 (↑ grains, vegetables, fruits, and legumes) 20 g CH ^f increase to 40 g	no no	3-d Food record	50 47	4
Davis and colleagues, 2009 ³³	105 36 100	53.5 75 25	1	LF vs LC	25 (rich in whole grains) 20-25 g CH for 2 wk, increase 5 g/wk (CH)	no no	24-h Recall	19 19	3

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Table 1. General characteristics of randomized controlled intervention trials included in the meta-analysis of studies assessing the long-term effects of low-fat (LF) diets compared with diets with high amounts of fat (HF) on blood lipid levels (*continued*)

Reference	Participants, BMI ^a , % diabetes	Age (y), women (%), men (%)	Duration (y)	Dietary Intervention	Dietary protocol fat (%), protein (%), carbohydrates (%) (source of carbohydrates)	Energy restricted (kcal)	Dietary assessment	Drop-out rate (%)	Study quality (Jadad score)
Ebbeling and colleagues, 2005 ³⁵	23 >27 0	28.5 96 4	1	LF vs LGI ^g	<30, 15, 55-60 (rich in whole grains) 30-35, 15, 45-50 LGI foods ^{85,86}	–250 to –500 no	Food diaries	29 35	2
Ebbeling and colleagues, 2007 ³⁴	73 nd 0	27.5 79 21	1,5	LF vs LGL ^h	20, 25, 55 (↑ grains, vegetables, fruits, and legumes) 35, 25, 40 LGI foods ⁸⁷	no no	3-d 24-h Recall	38 22	4
Elhayany and colleagues, 2010 ²⁶	259 31.4 100	56.3 48 52	1	LF ⁱ vs MUFA	30, 20, 50 (↑ grains, vegetables, fruits, and legumes) 45, 20, 35, 23 MUFA	1,700 1,700	24-h Recall, FFQ	32 28	2
Esposito and colleagues, 2009 ³⁶	215 29.6 100	52.2 51 49	4	LF vs MUFA	<30, 15, 55 (rich in whole grains) >30, 15, <50, 30-50 g/day olive oil	1,500 women, 1,800 men 1,500 women, 1,800 men	Diet diaries	9 9	4
Frisch and colleagues, 2009 ³⁷	200 33.5 nd	47 69 31	1	LF vs LGI	<30, 15, 55 (↑ grains, vegetables, fruits, and legumes) >35, 25, 40	–500 –500	3-d Food record	20 15	4
Foster and colleagues, 2003 ³⁸	63 34.2 0	44.1 68 32	1	LF vs LC	25, 15, 60 (↑ grains, vegetables, fruits, and legumes) 20 g CH increase to stable and desired weight	1,200-1,500 women 1,500-1,800 men no	nd	43 40	2

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Table 1. General characteristics of randomized controlled intervention trials included in the meta-analysis of studies assessing the long-term effects of low-fat (LF) diets compared with diets with high amounts of fat (HF) on blood lipid levels (*continued*)

Reference	Participants, BMI ^a , % diabetes	Age (y), women (%), men (%)	Duration (y)	Dietary Intervention	Dietary protocol fat (%), protein (%), carbohydrates (%) (source of carbohydrates)	Energy restricted (kcal)	Dietary assessment	Drop-out rate (%)	Study quality (Jadad score)
Foster and colleagues, 2010 ³⁹	307 36.1 0	45.5 68 32	2	LF vs LC	30, 15, 55 (↑ grains, vegetables, fruits, and legumes) 20 g CH for 12 wk, increase 5 g/wk CH	1,200-1,500 women, 1,500-1,800 men no	nd	32 42	3
Gardner and colleagues, 2007 ¹⁴	232 31.66 0	41.33 100	1	LF ^k vs LC	10/30 (↑ grains, vegetables, fruits, and legumes) 20 g CH	no/yes no	3-d, 24-h Recall	23 12	4
Guldbrand and colleagues, 2012 ⁴⁰	61 32.7 100	62 56 44	2	LF vs LC	<30, 10-15, 60 50, 30, 20	1,600 women, 1,800 men 1,600 women, 1,800 men	3-d Diet record	10 13	3
Hockaday and colleagues, 1978 ⁴¹	93 nd 100	51.5 56 44	1	LF vs control	26, 20, 54 (↑ complex carbohydrates) 40, 20, 40	1,500 1,500	nd	nd	1
Howard and colleagues, 2010 ⁴²	2730 nd 11	61.7 100	7.5	LF vs control	<20 (vegetable and fruit consumption ≥5 servings/d and grains ≥6 servings/d) >30	no no	FFQ ^l	5 4	3
Iqbal and colleagues, 2010 ⁴³	68 37.5 100	60 11 89	2	LF vs LC	<30 (↑ vegetable and fruit consumption) 30 g CH	—500 no	Self-reported dietary intake	40 54	4
Kasim and colleagues, 1993 ⁴⁴	72 nd 0	46 100	1	LF vs control	15 (↑ grains, vegetables, fruits, and legumes) >30	no no	3-d Food records	nd	1
Klemsdal and colleagues, 2010 ⁴⁵	202 nd nd	50 48 42	1	LF vs LGL ^m	30, 15, 55-60 (↑ unrefined, high fiber CH) 35-40, 25-30, 30-35	—500 —500	7-d Dietary questionnaire	23 23	2

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Table 1. General characteristics of randomized controlled intervention trials included in the meta-analysis of studies assessing the long-term effects of low-fat (LF) diets compared with diets with high amounts of fat (HF) on blood lipid levels (*continued*)

Reference	Participants, BMI ^a , % diabetes	Age (y), women (%), men (%)	Duration (y)	Dietary Intervention	Dietary protocol fat (%), protein (%), carbohydrates (%) (source of carbohydrates)	Energy restricted (kcal)	Dietary assessment	Drop-out rate (%)	Study quality (Jadad score)
Lanza and colleagues, 2001 ⁴⁶	744 27.55 nd	61.4 35 65	4	LF vs control	20 (fiber: 18 g/1,000 kcal) >30	no no	4-d Food record, FFQ, 24-h recall	2 12	2
Lee-Han and colleagues, 1998 ⁴⁷	57 nd nd	70 100	1	LF vs control	15 35-40	no no	4-d Food record	22 15	1
Ley and colleagues, 2004 ⁴⁸	103 29.2 22	52.3 26 74	5	LF vs control	<30 (↑ fiber intake) >30	no no	3-d Food diary	nd	1
Lim and colleagues, 2010 ²⁵	82 31.4 0	48.5 80 20	1.5	LF ⁿ vs LC	10/30, 20, 70/50 (↑ grains, vegetables, fruits, and legumes) 60, 35, 4	1,555 1,555	3-d Weighed food diary	40 37	3
McAuley and colleagues, 2006 ⁴⁹	48 nd insulin resistant	nd 100	1	LF vs LC	<30, 15, 55 (25-30 g fiber/d) 20 g CH for 2 wk, 50 g for 8 wk	8-wk WLP ^o 8-wk WLP	3-d Food diary	25 23	2
Milne and colleagues, 1994 ⁵⁰	43 29.5 100	59.5 53 47	1.5	LF vs control	30, 15, 55 (≥30 g fiber/d) 36, 19, 45	-500 kcal BMI >25 -500 kcal BMI >25	24-h Recall	nd	1
National Diet Heart Study, 1968 ²⁸	709 nd nd	nd 100	1	LF vs control	30 40	no no	7-d Food records	nd	3
Sacks and colleagues, 2009 ⁵¹	811 33 0	51 64 36	2	LF/LP ^p vs HF/LP (control) LF/HP ^q vs HF/HP	20, 15, ^r 65 (↑ grains, vegetables, fruits, and legumes) 40, 15, 45 ^s	-750 -750	24-h Recall at 3 nonconsecutive days	17 26 22 16	4

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Table 1. General characteristics of randomized controlled intervention trials included in the meta-analysis of studies assessing the long-term effects of low-fat (LF) diets compared with diets with high amounts of fat (HF) on blood lipid levels (*continued*)

Reference	Participants, BMI ^a , % diabetes	Age (y), women (%), men (%)	Duration (y)	Dietary Intervention	Dietary protocol fat (%), protein (%), carbohydrates (%) (source of carbohydrates)	Energy restricted (kcal)	Dietary assessment	Drop-out rate (%)	Study quality (Jadad score)
Shai and colleagues, 2008 ¹⁵	213 30.9 14	52 14 86	2	LF vs LC	<30 (↑ grains, vegetables, fruits, and legumes) 20 g CH increase to max 120 g	1,500 women, 1,800 men no	FFQ	10 22	4
Stern and colleagues, 2004 ⁵²	132 42.9 41	53.5 17 83	1	LF vs LC	<30 fat (rich in whole grains) <30 g CH	−500 no	Dietary recall data	37 31	3
White and colleagues, 1992 ⁵³	413 nd nd	nd 100	3	LF vs control	<20 >30	no no	4-d Diet records	15 12	2
Wolever and colleagues, 2009 ²⁷	156 30.9 100	59.86 54 46	1	LF ^t vs MUFA	<30 (rich in starchy CH) >35, ↑ fat intake by ~10 replacing CH (nuts, olive and canola oils)	−500 ^u −500 ^u	3-d Food record	20 19	3

^aBMI=body mass index.^bnd=no data.^cTwo kinds of LF diets that differed in the amount of fiber (15 g vs 50 g).^dMUFA=monounsaturated fatty acid.^eLC=low carbohydrate.^fCH=carbohydrate.^gLGI=low glycemic index.^hLGL=low glycemic load.ⁱTwo kinds of LF diets that differed in the amount of fiber (15 g vs 30 g).^jTwo kinds of LF diets (Ornish and Learn: 10% and 30% of total energy consumption).^kFFQ=food frequency questionnaire.^mGI diet: One third of the plate for all meals with a primary protein source (fish, fowl, red meat, cottage cheese, and eggs). Two meals daily the other two thirds of the plate consisted of vegetables, salads, and/or legumes. At the third meal the remaining two thirds was divided between CH, including bread or other LGI bread types.ⁿTwo kinds of LF diets (10% and 30% of total energy consumption).^oWLP=weight loss phase.^pLP=low protein.^qHP=high protein.^rTwenty-five percent of total energy consumption as protein in the LF/HP.^sThirty-five percent of total energy consumption as carbohydrates in the HF/HP.^tTwo kinds of LF diets (high glycemic index and LGI).^uIf subject wished to lose weight.

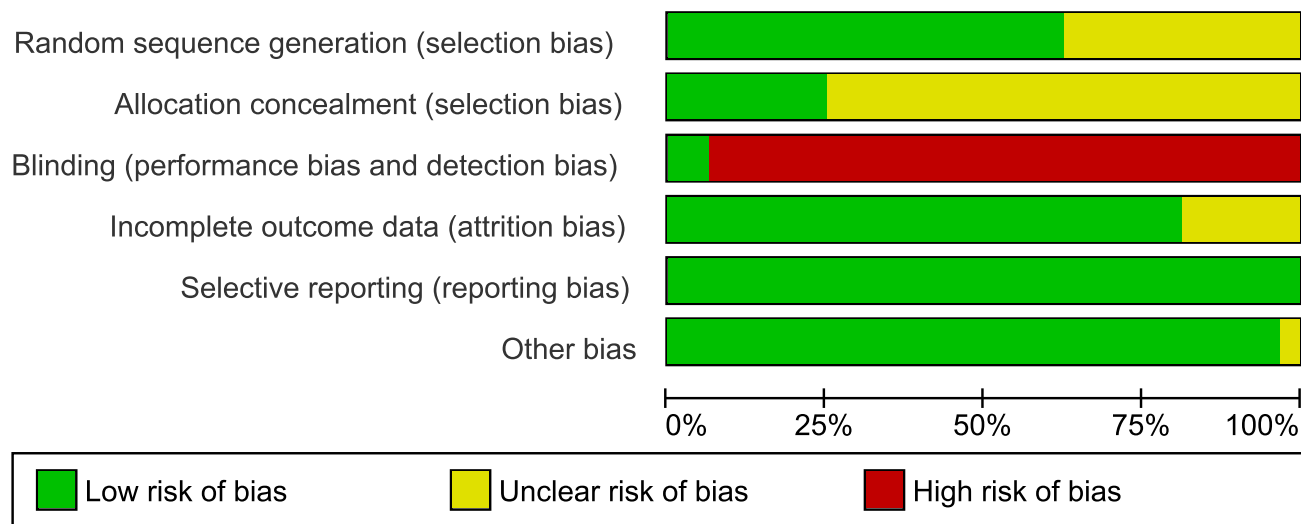


Figure 1. Risk of bias assessment tool. Data from trials assessing the long-term effects of low-fat diets compared with diets with high amounts of fat on blood lipid levels, by their conclusion of a low risk of bias (green), an unclear risk of bias (yellow), or from trials with high risk of bias (red).

Diet is accepted to be one of the cornerstones in the primary prevention of obesity and/or its associated detrimental consequences. Regarding the optimal macronutrient composition and especially the content of fat in the diet, recommendations by the Nutrition Committee of the American Heart Association⁹ give a reference value of <35% of total energy consumption in the form of fat and the 2010 edition of the Dietary Guidelines for Americans¹⁰ proposes a range of 20% to 35% of daily energy as fat. In contrast to these general recommendations, there is a plethora of contradictory guidelines for weight management, dietary treatment of obesity, and prevention of CVD and their corresponding effects on CVD risk factors with respect to percentage of dietary fat. As a general rule, these different dietary regimens can be categorized into low-fat (LF) (mostly ≤30% of total energy consumption) and high-fat (HF) (mostly >30% of total energy consumption) regimens and both are described by their proponents to be superior to their respective counterpart.^{11–15}

To pass an evidence-based judgment on the positive and negative aspects of LF and HF diets, their long-term (≥12 months) effects need to be analyzed. Our meta-analysis focused on these long-term effects of LF and HF diets on blood lipid levels in overweight and obese patients. The effects of typical HF diets like Atkins (LC), low glycemic index or low glycemic load (LGI/GL) diets, and high protein (HP) diets as well as dietary regimens rich in monounsaturated fat (MUFA) were investigated in comparison to LF regimens.

METHODS

The review protocol has been registered in PROSPERO International Prospective Register of Systematic Reviews (www.crd.york.ac.uk/prospero/index.asp). Identifier: CRD42012002742.

Literature Search

Queries of literature were performed using the electronic databases MEDLINE (until March 2013), EMBASE (until March 2013), and the Cochrane Trial Register (until March 2013) with restrictions to randomized controlled trials (RCTs) but no

restriction to language, using the following search terms: *low fat diet*, *high fat diet*, *low carbohydrate diet*, and *high carbohydrate diet*. Moreover, the reference lists from retrieved articles were checked to search for further relevant studies. This systematic review was planned, conducted, and reported in adherence to standards of quality for reporting meta-analyses.¹⁶ The literature search was conducted independently by both authors, with disagreements resolved by consensus.

Eligibility Criteria

Studies were included in the meta-analysis if they met all of the following criteria: randomized controlled design, minimum intervention period with a follow-up of 12 months, comparing an LF (≤30% of total energy consumption) with an HF dietary intervention (>30% of total energy consumption), assessment of the outcome of interest markers (ie, total cholesterol, LDL cholesterol, HDL cholesterol, total cholesterol:HDL cholesterol, and triglycerides), and report of post-intervention mean or mean of two time point values with standard deviation (or basic data to calculate these parameters). RCTs on secondary prevention of CVD were excluded. If data of ongoing studies were published as updates, results of only the longest duration periods were included.

Types of Intervention

The focus of our systematic review was set on examining the effects of LF compared with HF diets on values of blood lipid levels in overweight and/or obese patients following iso- and hypocaloric diets. The following types of dietary interventions were evaluated: as a primary analysis, LF was defined as providing ≤30% of total energy consumption in the form of fat,^{17,18} and HF as providing >30% of total energy consumption as fat. HF diets could be differentiated to alternative macronutrient combinations, with a special emphasis on fatty acid composition:

- Control diet (total fat >30% of total energy consumption, saturated fatty acids (SFAs) >10% of total energy

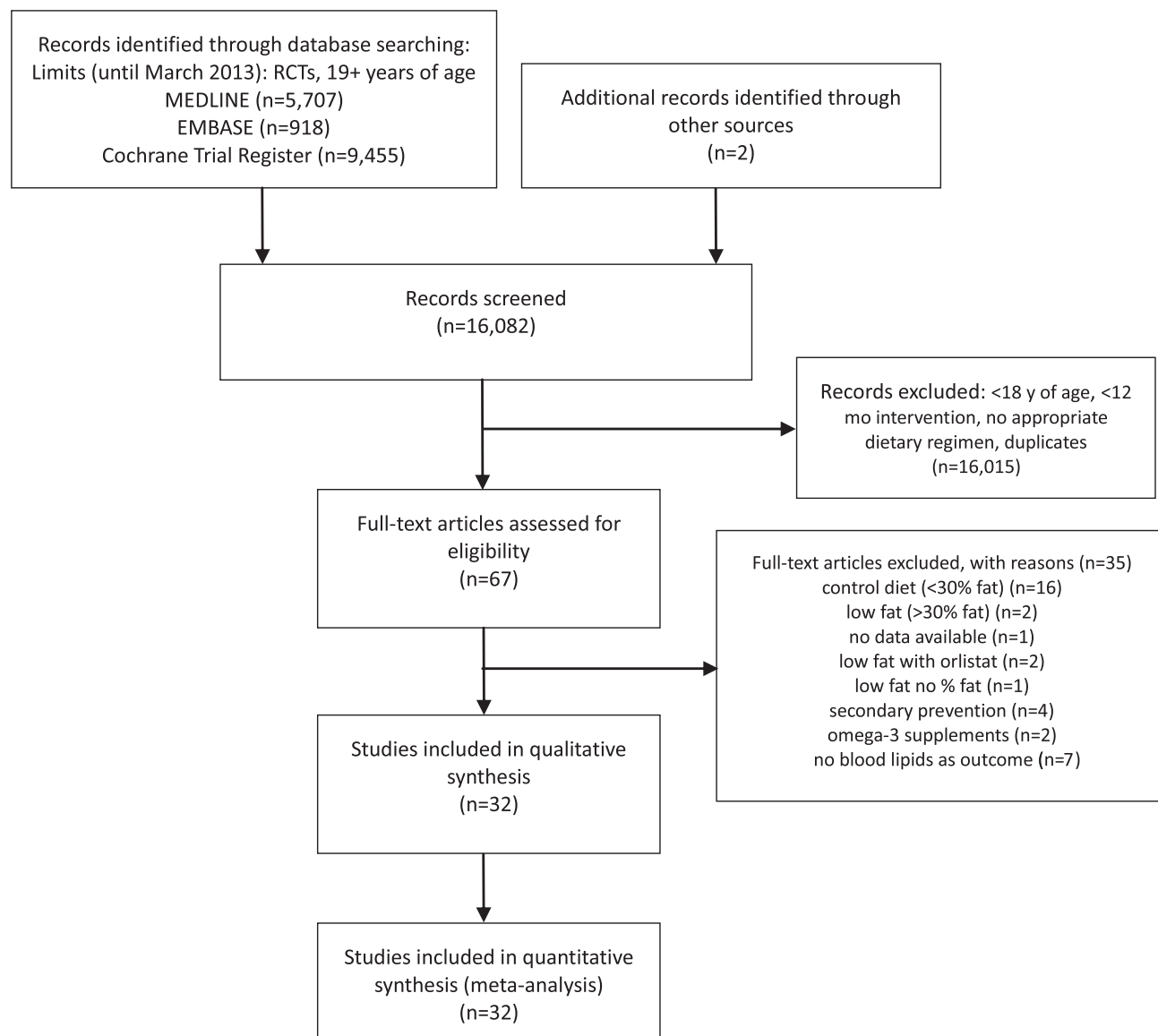


Figure 2. Flow chart for article selection process in our systematic review and meta-analysis focused on randomized controlled trials assessing the long-term effects of low-fat diets compared with diets with high amounts of fat on blood lipid levels.
^aRCTs=randomized controlled trials.

Table 2. Pooled estimates of effect size and 95% CIs expressed as weighted mean difference (WMD) for the effects of low-fat vs high-fat diets on blood lipid levels in 32 studies

Outcomes	No. of studies	Participants (n)	WMD (mg/dL ^a)	95% CI	P value	I ² (%)
Total cholesterol	29	8,042	−4.55	−8.03 to −1.07	0.01	82
Total cholesterol ^b	14	5,692	−6.08	−11.43 to −0.73	0.03	90
Low-density lipoprotein cholesterol	26	6,370	−3.11	−4.51 to −1.71	<0.0001	0
Low-density lipoprotein cholesterol ^b	14	5,327	−3.48	−5.01 to −1.94	<0.00001	0
High-density lipoprotein cholesterol	28	6,694	−2.35	−3.42 to −1.29	<0.0001	73
High-density lipoprotein cholesterol ^b	16	5,594	−2.06	−3.27 to −1.53	0.0008	79
Triglyceride	28	6,731	8.38	3.25 to 13.50	0.001	51
Triglyceride ^b	16	5,595	6.74	0.83 to 12.65	0.03	56
Total cholesterol: high-density lipoprotein cholesterol	5	709	0.17	0.00 to 0.33	0.05	4
Total cholesterol: high-density lipoprotein cholesterol ^b	4	606	0.12	−0.12 to 0.36	0.32	28

^aTo convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.6. To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.026. Cholesterol of 40 mg/dL=1.04 mmol/L. To convert mmol/L triglyceride to mg/dL, multiply mmol/L by 88.5. To convert mg/dL triglyceride to mmol/L by 0.0113. Triglyceride of 160 mg/dL=1.8 mmol/L.

^bSensitivity analysis including studies with a Jadad score ≥ 3 only.

consumption, and carbohydrates $\geq 40\%$ of total energy consumption),

- LC diet (total fat $>30\%$ of total energy consumption, SFA $>10\%$ of total energy consumption, and <50 g carbohydrate daily),
- LGL/LGI diet (total fat $>30\%$ of total energy consumption, carbohydrate $<50\%$ of total energy consumption, and LGL foods),
- HP diet (total fat $>30\%$ of total energy consumption, SFA $<8\%$ of total energy consumption, and total protein $\geq 25\%$ of total energy consumption), and
- high MUFA diet (total fat $>30\%$ of total energy consumption and MUFA $>12\%$ of total energy consumption).

Quality Assessment of Studies/Risk of Bias Assessment Tool

Full copies of studies were independently assessed for methodologic quality by both authors using the Jadad score.¹⁹ This 5-point quality scale awards points for randomization (randomized=1 point, table of random numbers or computer-generated randomization=an additional 1 point), double-blinding (double-blind=1 point, use of a placebo=additional 1 point), and follow-up (numbers and reasons for withdrawal in each group are stated=1 point) within the report of an RCT. An additional point was accepted if the analysis was by intention-to-treat to compensate for the fact that double-blinded study protocols are difficult to implement in dietary intervention studies. Final scores of 0 to 2 were considered low quality, whereas final scores ≥ 3 were regarded as representing studies of high quality (see Table 1). Assessment of methodologic quality was performed using the risk of bias assessment tool by the Cochrane Collaboration indicating the following bias domains: selection bias (random sequence generation, allocation concealment), performance/detection bias (blinding of participants and personnel/blinding of outcome assessment), attrition bias (incomplete data outcome), reporting bias (selective reporting), and other bias (Figure 1).²⁰

Data Extraction

The following data were extracted from each study: the first author's last name, publication year, study duration, retention and drop-out rate, dietary assessment method, participant's sex and age, body mass index (BMI), percent with diabetes, sample size, outcomes, and postintervention mean values or differences in mean of two time point values with corresponding standard deviation. Subsequently, a standardized data extraction form for this systematic review was created according to Avenell and colleagues.²¹

Statistical Analysis

For each outcome measure of interest, a meta-analysis was performed to determine the pooled effect of the intervention in terms of weighted mean differences between the postintervention (or differences in means) values of the LF and HF groups. Combining both the postintervention values and difference in means in one meta-analysis is an accepted method described by the Cochrane Collaboration.²² All data were analyzed using Review Manager 5.1

software (2011, Cochrane Collaboration). Heterogeneity among trial results was tested with a standard χ^2 test. The I^2 parameter was used to quantify any inconsistency: $I^2 = [(Q - df)/Q] \times 100\%$, where Q is the χ^2 statistic. A value for $I^2 > 50\%$ was considered to represent substantial heterogeneity.²³ To consider heterogeneity, the random-effects model was used to estimate weighted mean differences with 95% CIs. Forest plots were generated to illustrate the study-specific effect sizes along with 95% CIs. To evaluate considerable heterogeneity ($I^2 > 75\%$ to 100%) a post hoc random-effects meta-regression was performed to examine the association between age, sex, BMI, study duration, carbohydrates, protein, dietary cholesterol, dietary fiber, total fat, SFA, and MUFA, as well as polyunsaturated fatty acid (PUFA) and changes in total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels, respectively. The P values for differences in effects between the covariates were obtained using the metareg function of Stata version 12.0 (2011, StataCorp). Two-sided $P < 0.05$ was considered to be statistically significant. When available, macronutrient data (total fat, SFA, MUFA, PUFA, fiber, cholesterol, carbohydrates, and protein [all as percent total energy consumption]) were derived from a food frequency questionnaire, 24-hour dietary recalls, or 3- to 7-day dietary protocols instead of relying on the description of the dietary intervention regimens as described in the protocols. Funnel plots were used to assess potential publication bias (eg, the tendency for studies that yield statistically significant results to be more likely to be submitted and accepted for publication). To determine the presence of publication bias, we assessed the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors. A primary analysis of all studies was performed oriented toward the definition of LF and HF diets, followed by a subanalysis of the specific kind of the HF dietary intervention: control diet (SFA $>10\%$ of total energy consumption), LC diet (SFA $>10\%$ of total energy consumption and <50 g carbohydrates daily), LGL/LGI diet (carbohydrates $<50\%$ of total energy consumption and LGL foods), HP diet (SFA $<8\%$ of total energy consumption), and MUFA diet (MUFA $>12\%$ of total energy consumption). The trials included both hypocaloric and isocaloric dietary regimens.

Five studies^{14,24-27} included two types of LF diets (15 g vs 50 g fiber, 15 g vs 30 g fiber, total fat content 10% vs 30% of total energy consumption, and LGL vs high GI), and these diets were combined to one LF group as described in the Cochrane Handbook.²² In the study by Shai and colleagues¹⁵ the LF and LC branches were extracted for meta-analysis, whereas the MUFA group was discarded and reanalyzed in the sensitivity analysis because duplicate application of the LF data would not be appropriate. Likewise, in the Diet Heart Study,²⁸ only the LF and control branches were included in the meta-analysis, whereas both the PUFA and an additional HF intervention groups were excluded. Finally, in the study by Lim and colleagues²⁵ the controls were dropped and the LC group was incorporated because the baseline values of the controls were significantly lower compared with the baseline value of the combined intervention groups. Data extraction was conducted independently by both authors, with disagreements resolved by consensus.

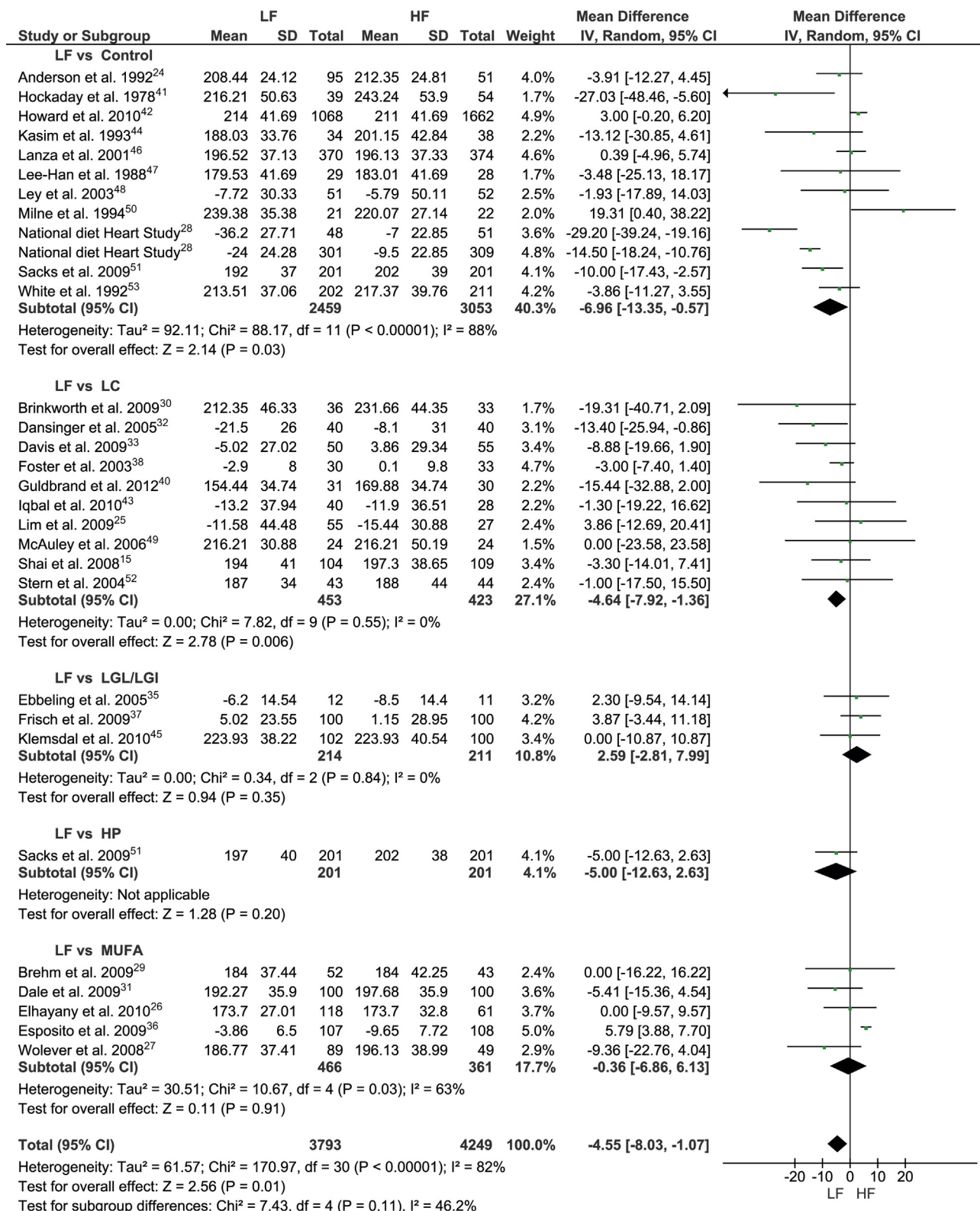


Figure 3.

Missing Data

Data processing for this review required the input of the mean and standard deviation of postintervention values or differences in means. When standard deviation was not available,²⁵ the standard deviation from the health technology assessment article by Avenell and colleagues²¹ (linear regression of 204 trials) was imputed.

RESULTS

Literature Search

A total of 32 studies extracted from 16,082 articles reviewed met the inclusion criteria and were analyzed in the systematic review.^{14,15,24-53} The detailed steps of the meta-analysis article selection process are given as a flow diagram in Figure 2.

Characteristics of Studies and Participants

All studies included in our meta-analysis were RCTs with a duration ranging between 12 months and 6 years, published between 1968 and 2012 (Table 1) and enrolling a total of 8,862 participants (restricted to patients aged 19 years and older). All studies included at least one type of LF dietary regimen. In 11 studies, this was compared with a control group, in 12 studies with an LC diet, in four studies with an LGL/LGL regimen, in five studies with a diet rich in MUFA, and in one study with an HP regimen. In case of more than one LF group within a single study design, all LF interventions were combined as recommended by the Cochrane Collaboration.²² In case of more than one comparison group (eg, a control group plus an independent HF regimen) without an eligible subgroup, all but one single comparison group was left out of consideration to avoid duplicate LF data in the meta-analysis. Studies strictly composed of participants with manifested CVD were excluded as well. The study by Shai and colleagues¹⁵ was admitted because only 34% of its participants had CVD. Reported BMI was >25 in all studies, and the mean age of participants varied between 27.5 and 62.3 years. No statistically significant differences between group drop-out rates were observed (LF: 22%, HF: 24%).

Weighted mean difference in change of body weight was not significantly different when comparing LF vs HF diets (−0.15 kg, 95% CI −0.80 to 0.50; $P=0.65$). However, when including only hypocaloric diets, there was a significantly greater decrease in body weight in the HF group compared with the LF counterparts (weighted mean difference −0.59 kg, 95% CI −1.04 to −0.15; $P=0.009$).

Blood Lipid Levels

The pooled estimate of effect size for the effects of LF compared with HF (~12.8% of total energy consumption as SFA, ~15.4%

of total energy consumption as MUFA, and ~8.3% of total energy consumption as PUFA) on blood lipid levels is summarized in Table 2. Weighted mean difference in change of total cholesterol was −4.55 mg/dL (−0.12 mmol/L) (95% CI −8.03 to −1.07; $P=0.01$) in favor of the LF diet (Figure 3). Subgroup analysis revealed a significant benefit of LF vs LC (SFA ~15% of total energy consumption) and control diets (SFA ~12.5% of total energy consumption), the corresponding weighted mean difference was −4.64 mg/dL (−0.12 mmol/L) (95% CI −7.92 to −1.36; $P=0.006$ and −6.96 mg/dL (−0.18 mmol/L) (95% CI −13.35 to −0.57; $P=0.03$).

Decreases in LDL cholesterol (Figure 4) were more explicit in the LF group than in the HF groups (with ~13% total energy consumption in the form of SFA) weighted mean difference −3.11 mg/dL (−0.08 mmol/L) (95% CI −4.51 to −1.71; $P<0.0001$). The subgroup analyses showed a more pronounced decrease in the LF group compared with the control groups (with ~12% of total energy consumption in the form of SFA) weighted mean difference −3.58 mg/dL (−0.09 mmol/L) (95% CI −6.60 to −0.56; $P=0.02$) as well as in comparison with the LC regimen (with ~15% of total energy consumption in the form of SFA) weighted mean difference −3.59 mg/dL (−0.09 mmol/L) (95% CI −6.29 to −0.88; $P=0.009$).

LF diets were associated with a decrease in HDL cholesterol compared with the HF regimen (~12.8% of total energy consumption as SFA, ~15.4% of total energy consumption as MUFA, ~8.3% of total energy consumption as PUFA) weighted mean difference −2.35 mg/dL (−0.06 mmol/L) (95% CI −3.42 to −1.29; $P<0.0001$). Subgroup analyses yielded a significant decrease in HDL cholesterol level following LF diets when comparing them with LC interventions (~15% of total energy consumption as SFA, ~17.5% of total energy consumption as MUFA, and ~7.3% of total energy consumption as PUFA) weighted mean difference −3.84 mg/dL (−0.10 mmol/L) (95% CI −5.26 to −2.42; $P<0.00001$) (Figure 5).

Weighted mean difference for the effects of LF vs HF diets on triglyceride level was 8.38 mg/dL (0.095 mmol/L) (95% CI 3.25 to 13.50), reflecting a significantly stronger increase due to LF ($P=0.001$) (Figure 6). Subgroup analysis showed detrimental effects of LF diets vs LC diets (~15% of total energy consumption as SFA, ~16.5% of total energy consumption as MUFA, and ~7% of total energy consumption as PUFA) weighted mean difference 12.20 mg/dL (0.14 mmol/L) (95% CI 5.19 to 19.22; $P=0.0006$). In addition, LF diets significantly raise triglyceride levels in comparison with LGL/LGL (~34.7% of total energy consumption as total fat and ~11.6% of total energy consumption as SFA) and MUFA regimens (~10% of total energy consumption as SFA, ~17% of total energy consumption as MUFA, and ~11% of total energy consumption as PUFA) weighted mean difference 8.41 mg/dL (0.095 mmol/L) (95% CI 0.68 to 16.13; $P=0.03$) and weighted mean difference

Figure 3. Forest plot showing pooled weighted mean difference with 95% CI for total cholesterol (mg/dL³) for 29 randomized, controlled low-fat (LF) diet studies. The different types of high-fat (HF) diets were separated into subgroups. For each LF study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of these effects. Arrows at the end of some of the 95% CIs are due to greater standard deviation (SD) and smaller sample size of the respective trial and were applied to increase readability of the effect size. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean difference with the 95% CI. To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.6. To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.026. Cholesterol of 40 mg/dL=1.04 mmol/L. IV=inverse variance. LC=low carbohydrate. LGL/LGI=low glycemic load/low glycemic index. HP=high protein. MUFA=monounsaturated fatty acids.

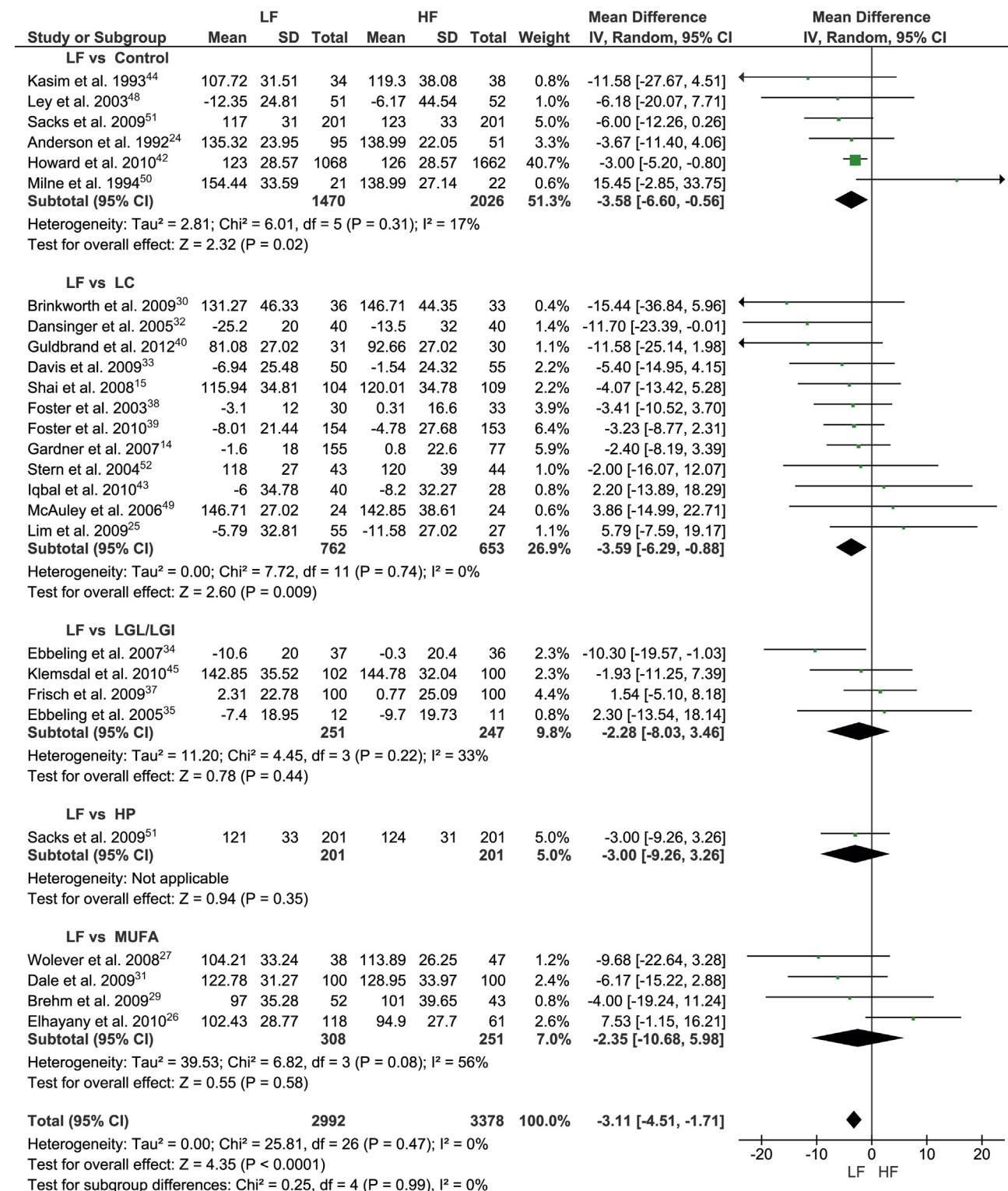


Figure 4.

12.79 mg/dL (0.14 mmol/L) (95% CI 2.16 to 23.41; $P=0.02$), respectively.

Increase of total cholesterol:HDL cholesterol was more explicit following LF diets compared with HF protocols ($\sim 12.6\%$ of total energy consumption as SFA, $\sim 15\%$ of total energy consumption as MUFA, and $\sim 6\%$ of total energy consumption as PUFA) weighted mean difference 0.17 mg/dL (95% CI 0.00 to 0.33; $P=0.05$). Analyses of subgroups illustrated a greater increase of total cholesterol:HDL cholesterol after LF diets when compared with LC counterparts ($\sim 13\%$ of total energy consumption as SFA, $\sim 15\%$ of total energy consumption as MUFA, and $\sim 5.5\%$ of total energy consumption as PUFA) weighted mean difference 0.23 mg/dL (95% CI 0.06 to 0.41; $P=0.009$).

Sensitivity Analyses

Following exclusion of articles with a Jadad quality score <2 , a total of 17 studies remained for sensitivity analyses.^{14,15,25,27,28,31-34,36,37,39,40,42,43,51,52} In summary, for all but one blood lipid parameter (ie, total cholesterol:HDL cholesterol), the results of the primary analyses could be confirmed (see Table 2). Likewise, results of the primary analyses were not altered following exclusion of patients with type 2 diabetes (data not shown). In addition, the largest study⁴² was excluded to account for a potential large study effect on the pooled weighted mean difference. However, the results were not significantly different compared with the comprehensive meta-analyses. Inclusion or exclusion of the study by Shai and colleagues¹⁵ (because of 34% of participants with CVD) did not change the level of significance. Taking account only of studies adopting an energy-restricted diet, meta-analyses still revealed a significant increase in HDL cholesterol level as well as a significant reduction in triglyceride levels, both in favor of HF regimens ($\sim 13\%$ of total energy consumption as SFA, $\sim 17\%$ of total energy consumption as MUFA, and $\sim 9.8\%$ of total energy consumption as PUFA). However, changes in total cholesterol and LDL cholesterol plasma concentrations were now found to be similar in LF ($\sim 9.7\%$ of total energy consumption as SFA, $\sim 10.8\%$ of total energy consumption as MUFA, and $\sim 7\%$ of total energy consumption as PUFA) and HF groups, whereas both biomarkers were affected in a beneficial fashion by LF diets when all studies were included in the meta-analysis. This was not affected by either inclusion or exclusion of patients with type 2 diabetes.

Publication Bias

The funnel plots (with respect to effect size changes for total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and total cholesterol:HDL cholesterol in response to LF diets) indicated little to moderate asymmetry, suggesting that

publication bias cannot be completely excluded as a factor of influence on our meta-analysis (data not shown). It remains possible that small studies yielding inconclusive data have not been published.

Heterogeneity

High heterogeneity was found with respect to total cholesterol ($I^2=82\%$ and 90%) and HDL cholesterol ($I^2=73\%$ and 79%) in both primary and sensitivity analyses, respectively. It was assumed that high heterogeneity might be explained by nonuniform study characteristics in the HF groups; for example, by variations in postintervention macronutrient intake. Therefore, a random-effects meta-regression was performed to examine the associations between LF and HF group parameters and changes in total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, respectively. A statistically significant dose-response relationship was detected between SFA (direct) and PUFA (inverse) intake and increase in total cholesterol (Figure 7, A and B), between total fat (direct) and MUFA (direct) intake and increase in HDL cholesterol (Figure 7, C and D) and between carbohydrate (direct) intake and rise in triglyceride level (Figure 7, E). In addition, LDL cholesterol was marginally associated ($P=0.074$) with higher intakes of SFA (data not shown).

DISCUSSION

Potential Benefits of LF Diets

Dietary interventions represent a useful tool in the prevention of obesity as well as its associated diseases such as CVD. With respect to macronutrient composition, the recommendations for dietary measures in international literature are often confusing and sometimes even contradictory. This specifically applies to the proposed percentage of fat in nutrition regimens. In 2003, a systematic review and meta-analysis concluded that the lack of long-term data on LC diets significantly limits the understanding of the efficacy and safety of these interventions.⁵⁴ This statement is applicable to the comparison of LF and HF diets as well. One major finding of our meta-analysis is that LF diets ($\sim 9.3\%$ of total energy consumption in the form of SFA) resulted in a quantitatively greater reduction of total cholesterol and LDL cholesterol in the long term when compared with HF diets (ranging from high SFA to high unsaturated fat). The subgroup analysis showed that the favorable effects on total cholesterol and LDL cholesterol levels were observed only when comparing LF with control and LC diets (both high in SFA; ie, $\sim 12.5\%$ to 15% of total energy consumption). Both total cholesterol and especially LDL cholesterol are well-established risk factors in the prognosis of CVD development.^{55,56} Thus, RCTs have shown that a 38.6 mg/dL (1 mmol/L) reduction in total

Figure 4. Forest plot showing pooled weighted mean difference with 95% CI for low-density lipoprotein cholesterol (mg/dL^a) of 26 randomized controlled low-fat (LF) diet studies. The different types of high-fat (HF) diets were separated into subgroups. For each LF study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of these effects. Arrows at the end of some of the 95% CIs are due to greater standard deviation (SD) and smaller sample size of the respective trial and were applied to increase readability of the effect size. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean difference with the 95% CI. To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.6. To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.026. Cholesterol of 40 mg/dL = 1.04 mmol/L. IV = inverse variance. LC = low carbohydrate. LGL/LGI = low glycemic load/low glycemic index. HP = high protein. MUFA = monounsaturated fatty acids.

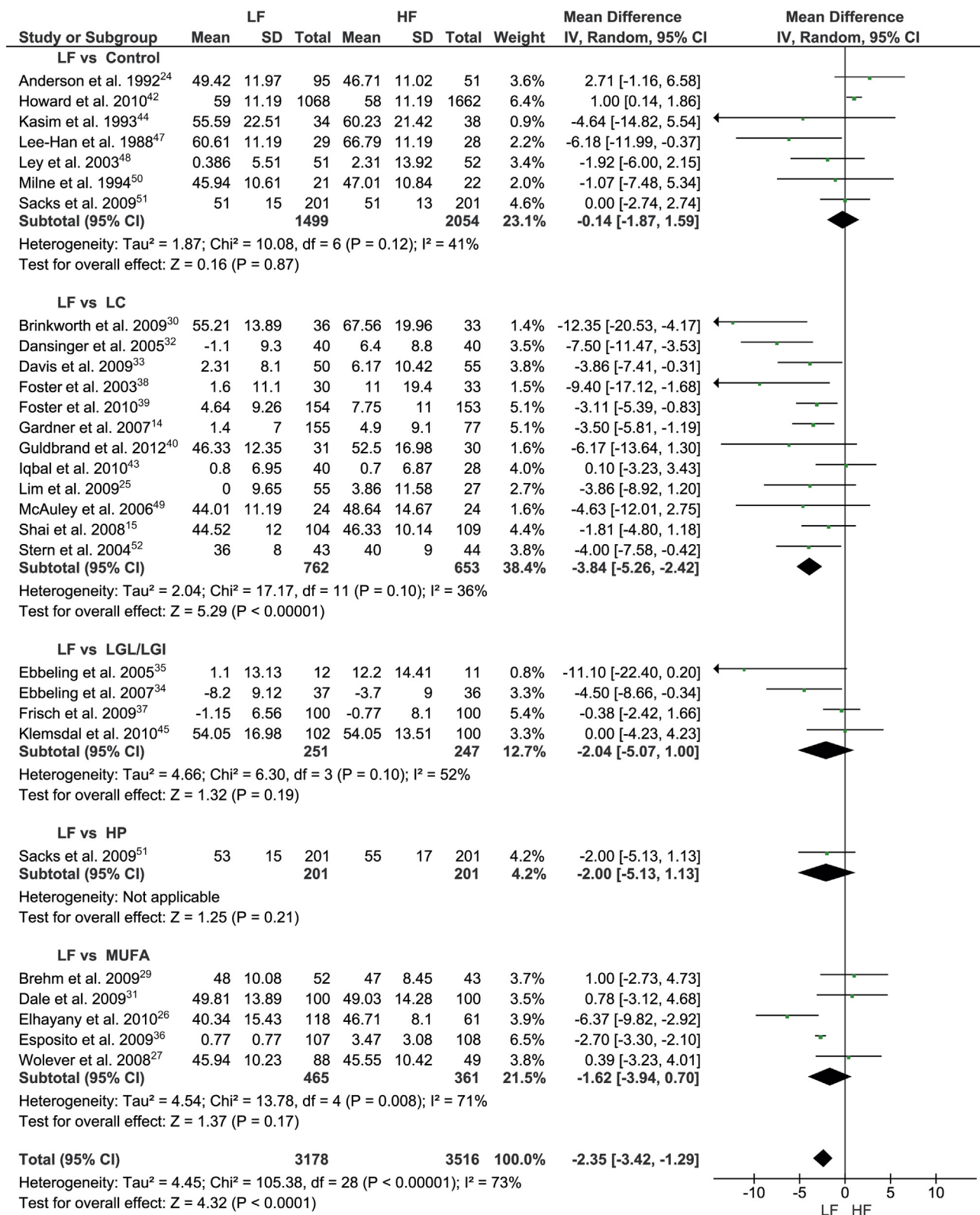


Figure 5.

cholesterol and LDL cholesterol levels resulted in a 15.6% to 17.5% decrease in relative risk for all-cause mortality, 24.5% to 28% for CVD-related mortality, and 26.6% to 29.5% for any CVD event.⁵⁷ The average reduction of LDL cholesterol level following the LF dietary interventions included in this meta-analysis was ~ 3 mg/dL (~ 0.08 mmol/L), which would correspond to a decrease in CVD risk of about 2% to 2.5%.

Controversial Results

In contrast to these benefits, LF diets increased triglyceride and decreased HDL cholesterol levels in a more profound manner compared with HF regimens. Considering HDL cholesterol, each 10-mg/dL increase is associated with a decreased coronary heart disease risk of 29%.⁵⁸ In relation to our meta-analysis, where HF diets yielded an average increase in plasma HDL cholesterol concentrations of 2.35 mg/dL (0.06 mmol/L), this would indicate a CVD risk reduction of 7%. Low levels of HDL cholesterol are regarded to be a residual risk modifier for the development of CVD following a successful reduction of LDL cholesterol plasma concentrations.⁵⁹ Thus, there seems to be compelling evidence for targeting both LDL cholesterol and HDL cholesterol to reduce CVD risk. On the other hand, pharmacologic strategies to raise HDL cholesterol levels have provided controversial results,⁶⁰ stressing the fact that HDL particle functionality might be at least as important as HDL cholesterol plasma levels. Elevated triglyceride levels are univariate predictors of CVD⁶¹ with an 88 mg/dL (1 mmol/L) increase being associated with a 14% increase of CVD risk in men and a 37% increase in women.⁶² In a recent meta-analysis,¹⁷ comparison of regimens providing higher amounts of fat with LF diets yielded improvements in HDL cholesterol and triglyceride levels, but at the same time increases in LDL cholesterol and total cholesterol levels were seen following the HF regimens as well. Other systematic reviews of RCTs comparing LC vs LF diets reported inconsistent effects on total cholesterol and HDL cholesterol levels, significant improvements in triglyceride levels, but detrimental effects on LDL cholesterol levels.^{63,64} In patients with type 2 diabetes, an LF diet increased triglyceride levels and decreased HDL cholesterol levels when compared with an LC/HF diet.⁶⁵ However, these meta-analyses did not investigate the differential influence of distinct types of fatty acids on lipid levels.

Potential Confounding Variables

Carbohydrate Content in LF Diets. Apart from the lipids mentioned, lipoprotein particle size represents another biomarker of CVD risk with smaller, denser LDL cholesterol particles being a predictor of the onset of CVD.⁶⁶ SFA has been shown to induce elevations in larger LDL cholesterol particles,⁶⁷ whereas carbohydrate-rich diets have been shown to

increase dense LDL cholesterol particles.⁶⁸ It seems reasonable to assume that LF diets are reciprocally high in carbohydrates and vice versa. Variations of carbohydrate content in the different dietary regimens may account for at least some of the changes in blood lipid levels found in our meta-analyses. However, except for triglyceride levels, changes in biomarkers were not associated with dietary carbohydrate content in our study (data not shown). In addition, sugar and other unrefined carbohydrates cannot be made responsible for any of the detrimental effects of LF diets found in our meta-analysis because a high carbohydrate load was realized using high molecular carbohydrates in the included RCTs (Table 1). Therefore, variations in dietary carbohydrate content most likely cannot explain the differences in LDL cholesterol or HDL cholesterol responses observed following LF and HF diets in this systematic review.

Fat Composition in HF Diets. In contrast, the results of the meta-regressions support the hypothesis that the remarkable heterogeneity with regard to total cholesterol and HDL cholesterol might be explained by a wide range in the HF diets for total fat (>30% to 60%), SFA, MUFA, and PUFA content. In brief, increases in total cholesterol levels were associated with higher SFA and lower PUFA contents of HF regimens, whereas a rise in HDL cholesterol level was related to higher amounts of total fat with an emphasis on MUFA content. In congruence with these observations, a meta-analysis of short-term studies suggested that LF diets are more likely to result in higher triglyceride levels and lower HDL cholesterol levels than diets where SFA are completely replaced by unsaturated fatty acids.⁶⁹ When comparing LF regimens with a Mediterranean-style diet (ie, high in MUFA), cardiovascular risk factors and markers of vascular inflammation were more improved in individuals allocated to a Mediterranean diet.⁷⁰⁻⁷³ In addition, a recent review of 16 meta-analyses⁷⁴ indicated that a diet rich in MUFA has several beneficial effects on a broad range of CVD risk factors, not only in the primary prevention of CVD. According to Mozaffarian and colleagues,⁷⁵ consumption of PUFA instead of SFA was suggested to reduce CVD events, and modification of dietary fat without reducing total fat intake was associated with a small but significant fall in cardiovascular risk.⁷⁶ In this context, the choice of PUFA might be of special importance as indicated by recently published data from the Sydney Diet Heart Study.⁷⁷ The authors reported a significant increase in the rate of death from CVD, coronary heart disease, and all-cause mortality in participants replacing SFA with n-6 PUFA. The observational literature is not consistent regarding self-reported SFA intakes and risk of CVD/coronary heart disease. Recent studies have shown that SFA intakes were not associated with changes in coronary heart disease, stroke,

Figure 5. Forest plot showing pooled weighted mean difference with 95% CI for HDL cholesterol (mg/dL³) of 28 randomized controlled low-fat (LF) diet studies. The different types of high-fat (HF) diets were separated into subgroups. For each LF study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of these effects. Arrows at the end of some of the 95% CIs are due to greater standard deviation (SD) and smaller sample size of the respective trial and were applied to increase readability of the effect size. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean difference with the 95% CI. To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.6. To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.026. Cholesterol of 40 mg/dL = 1.04 mmol/L. IV = inverse variance. LC = low carbohydrate. LGL/LGI = low glycemic load/low glycemic index. HP = high protein. MUFA = monounsaturated fatty acids.

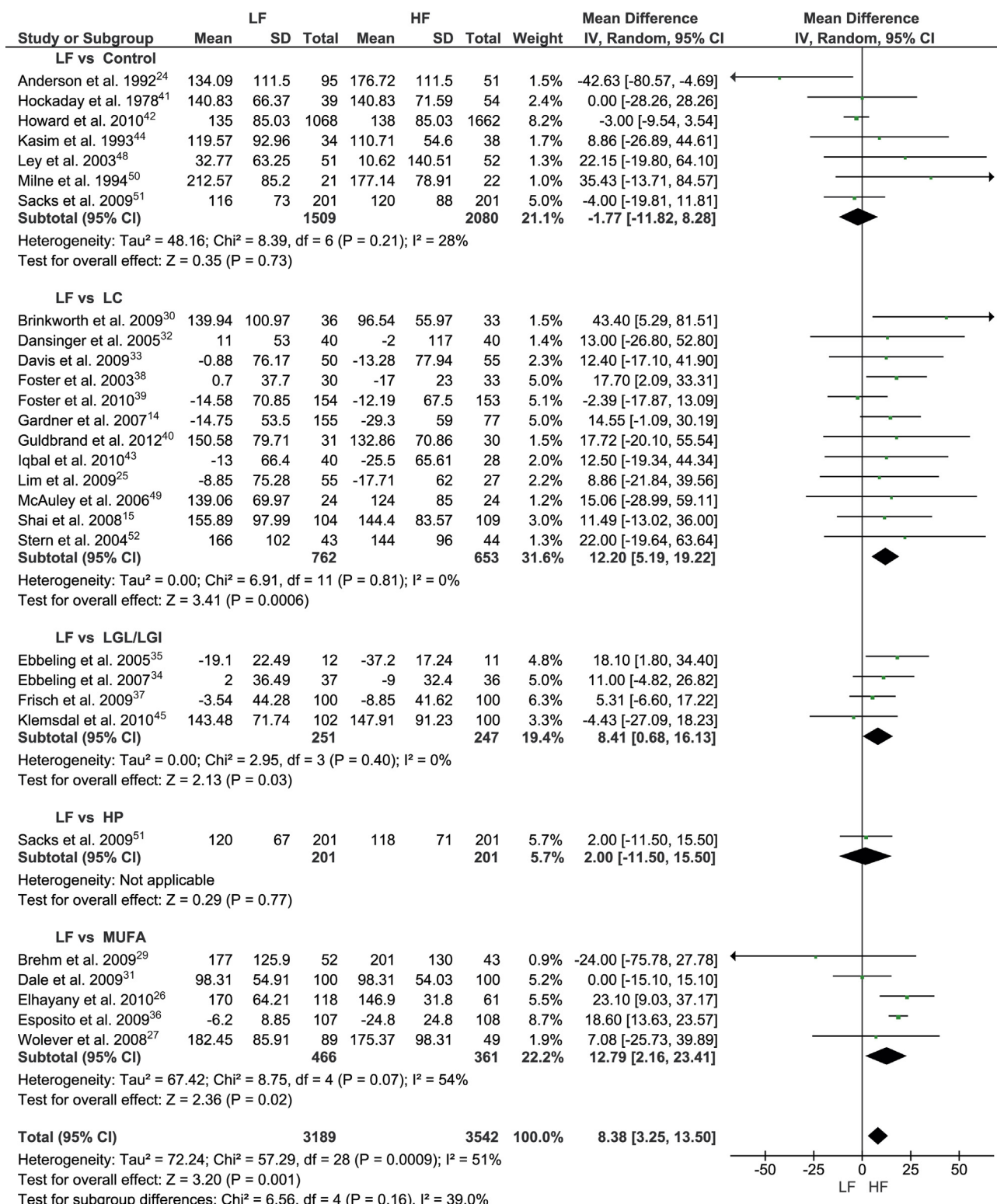


Figure 6.

or CVD frequencies,⁷⁸⁻⁸¹ although in one of these trials, increased MUFA intake was associated with a significantly lower risk of coronary heart disease.⁸¹ In contrast, the Framingham studies demonstrated that self-reported SFA is predictive of coronary heart disease in men.⁸² Nevertheless, the National Lipid Association 2011 Fatty Acid Summit concluded that SFAs have harmful effects on LDL cholesterol and are probably equivalent to high-GI carbohydrates with respect to CVD risk.⁸³

Weight Changes in Both Dietary Protocols. Weight change might represent a potential confounder in the interpretation of blood lipid levels. Thus, in a meta-analysis by Dattilo and Kris-Etherton,⁸⁴ each kilogram of weight loss was associated with a 1.93 mg/dL (0.05 mmol/L) decrease in total cholesterol and a 0.77 mg/dL (0.02 mmol/L) decrease in LDL cholesterol level, respectively. The data of our systematic review indicated no significant differences in weight change between LF and HF diets. This observation was no longer valid following a sensitivity analysis, including only trials adopting a hypocaloric diet. Decreases in body weight were significantly more pronounced in participants following an HF regimen compared with an LF diet. This may explain the fact that LF diets do not exert any beneficial effects on total cholesterol and LDL cholesterol levels in studies aiming specifically at weight loss (in contrast to the observations made when all studies were included in our meta-analyses).

Limitations of Our Systematic Review

Our systematic review does not consider unpublished data, and it cannot be excluded that these results may have had at least a moderate effect on the effect size estimates. Examination of funnel plots showed moderate asymmetry suggesting that publication bias cannot be completely excluded as a confounder of our meta-analysis (eg, lack of published studies with inconclusive results). A major limitation of nutrition intervention trials is the heterogeneity of various aspects and characteristics of the study protocols. Therefore, it is not surprising that the RCTs included in our analysis varied regarding type(s) of diets used (ie, energy restriction or isocaloric), definitions of LF and HF diets, study population (ie, patients with overweight, obesity, type 2 diabetes, abnormal glucose metabolism, large bowel syndrome, or breast dysplasia), intervention time, and nutritional assessment as well as long-term follow-up (1 to 6 years). Following sensitivity analyses including only studies adopting a hypocaloric diet, the beneficial effects of LF diets on total cholesterol as well as LDL cholesterol levels were not present anymore, indicating weight loss in the HF group to be a probable confounder. However, changes in triglyceride and HDL cholesterol levels were still significantly more pronounced in favor of an HF dietary

regimen. With respect to other potential modulating variables, sensitivity analyses and meta-regressions failed to show any correlations between the findings of the meta-analyses and age, sex, BMI, study duration, and manifested type 2 diabetes (data not shown). Not all of the studies provided information on the quality of their respective setup (eg, method of randomization or follow-up protocol with reasons for withdrawal), demanding a conservative interpretation of results. However, when sensitivity analyses were performed, including studies with a Jadad score ≥ 3 only, results remained statistically significant for all but one blood lipid parameter; that is, total cholesterol:HDL cholesterol, which was therefore omitted from discussion and interpretation.

Participant adherence is addressed in Table 1. With average values of 22% for LF and 24% for HF diets, respectively, drop-out rates can be regarded as low. However, this does not imply that adherence to a given study protocol was high enough as well. In the study by Sacks and colleagues,⁵¹ assessment of nutrient intakes provided evidence that participants did not reach the target values for macronutrients resulting in only minimal differences between the study arms. In this context it should be noted that the RCTs varied with respect to dietary assessment methods (Table 1) to validate compliance as well as individual intakes. Because every nutritional assessment method has its benefits and flaws, the method chosen for evaluation was not subjected to a quality score. Some of our meta-analyses were done using both postintervention values and changes in mean difference, which was considered an acceptable procedure as described by the Cochrane Collaboration.²²

CONCLUSIONS

The major finding of our meta-analysis of long-term RCTs was that LF diets decreased LDL cholesterol levels in a more pronounced fashion when compared with their HF counterparts. On the other hand, HF diets (ranging from high SFA to high unsaturated fat) were superior to LF diets with respect to HDL cholesterol plasma concentrations. With respect to dietary recommendations in primary and secondary prevention of CVDs, these results seem to be controversial, because the ideal dietary regimen for reducing the risk of CVD would be one that lowers LDL cholesterol level and raises HDL cholesterol level simultaneously. The evidence that increased levels of LDL cholesterol cause CVD is impressive. However, the role of decreased HDL cholesterol as an isolated risk factor is still discussed controversially. Because increases in HDL cholesterol level might be achieved by lifestyle factors such as regular physical exercise, one might speculate that the LDL cholesterol level-reducing effect of LF diets as shown in our meta-analysis has a stronger emphasis on the associated CVD risk compared with the

Figure 6. Forest plot showing pooled weighted mean difference with 95% CI for triglycerides (mg/dL³) of 28 randomized controlled low-fat (LF) diet studies. The different types of high-fat (HF) diets were separated into subgroups. For each LF study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of these effects. Arrows at the end of some of the 95% CIs are due to greater standard deviation (SD) and smaller sample size of the respective trial and were applied to increase readability of the effect size. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean difference with the 95% CI. To convert mmol/L triglyceride to mg/dL, multiply mmol/L by 88.5. To convert mg/dL triglyceride to mmol/L, multiply mg/dL by 0.0113. Triglyceride of 160 mg/dL=1.8 mmol/L. IV=inverse variance. LC=low carbohydrate. LGL/LGI=low glycemic load/low glycemic index. HP=high protein. MUFA=monounsaturated fatty acids.

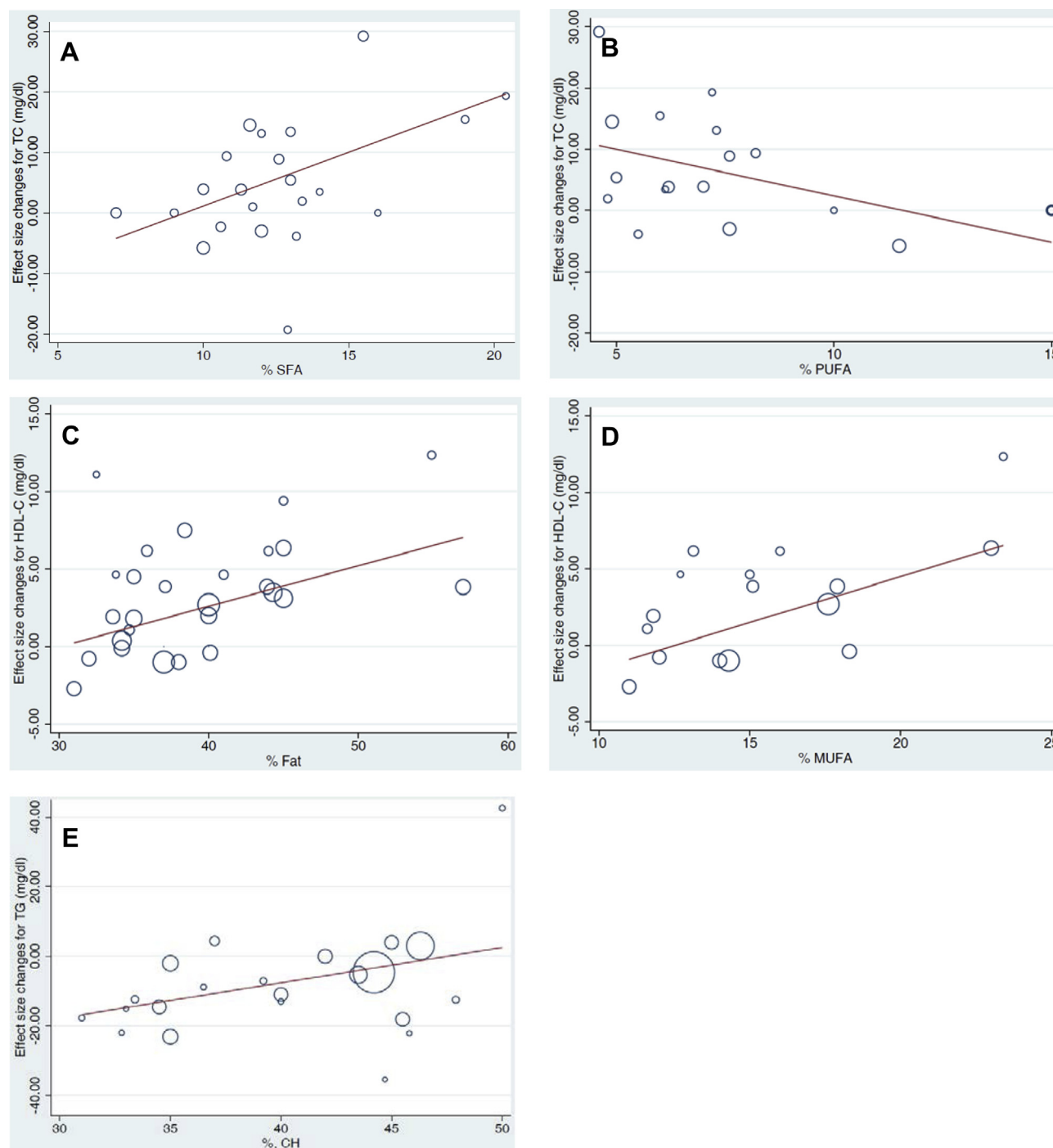


Figure 7. Bubble plots showing the dose–response relationship between intake of (A) saturated fatty acid (SFA) on changes in total cholesterol (TC) ($P=0.026$), (B) polyunsaturated fatty acid (PUFA) on changes in TC ($P=0.035$), (C) total fat on changes in high-density lipoprotein cholesterol (HDL-C) ($P=0.004$), (D) monounsaturated fatty acid (MUFA) on changes in HDL-C ($P=0.007$), and (E) carbohydrates (CH) on changes in triglyceride (TG) level ($P=0.019$). Macronutrient intake is given as percent of total energy consumption. The fitted regression lines together with the bubbles represent the estimates from each study, sized according to the precision of each estimate as calculated in the fitted random-effects meta-regression.

corresponding ineffectiveness regarding HDL cholesterol level. Based on the heterogeneity of RCTs included in our review, further research in the form of long-term interventional studies should adopt a more precise classification of

both LF (eg, <30% of total energy consumption) and HF (eg, 40%±5% of total energy consumption) measures, including specifications of total energy content as well as composition of other macronutrients such as carbohydrates. This might be

helpful in finding not only a recommendation with respect to the percentage of fat in daily nutrition, but also with respect to the composition of fat.

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Systematic review and meta-analysis

Comparison of the long-term effects of high-fat v. low-fat diet consumption on cardiometabolic risk factors in subjects with abnormal glucose metabolism: a systematic review and meta-analysis

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Abstract

The aim of the present systematic review and meta-analysis was to examine the long-term effects (≥ 12 months) of high-fat (HF) v. low-fat (LF) diet consumption on the indicators of glycaemic control as well as cardiovascular risk factors in pre-diabetic and diabetic individuals. Literature search was carried out using the electronic databases MEDLINE, Embase and the Cochrane Trial Register until November 2013. Study-specific weighted mean differences (MD) were pooled using a random-effects model of the Cochrane software package Review Manager 5.1 and Stata 12.0 was used for meta-regressions. A total of fourteen trials met the inclusion criteria and a maximum of 1753 subjects were included in the meta-analysis. HF regimens were found to result in a significant decrease in TAG levels (MD -0.19 mmol/l, 95% CI -0.23 , -0.14 , $P < 0.001$; $I^2 = 0\%$, $P = 0.58$) and diastolic blood pressure (MD -1.30 mmHg, 95% CI -1.73 , -0.87 , $P < 0.001$; $I^2 = 0\%$, $P = 0.60$) and a significant increase in HDL-cholesterol levels (MD 0.05 mmol/l, 95% CI 0.01 , 0.08 , $P = 0.01$; $I^2 = 57\%$, $P = 0.01$). In addition, MD in the reductions of fasting glucose levels (-0.41 mmol/l, 95% CI -0.74 , -0.08 , $P = 0.01$; $I^2 = 56\%$, $P = 0.02$) were significantly high in patients with type 2 diabetes adhering to a HF diet. HF and LF diets might not be of equal value in the management of either pre-diabetes or type 2 diabetes, leading to emphasis being placed on the recommendations of HF diets.

Key words: Low-fat diets: High-fat diets: Meta-analyses: Systematic review: Abnormal glucose metabolism

With an estimated prevalence of 350 million cases worldwide, diabetes represents one of the most serious and pressing current health problems. Type 2 diabetes accounts for approximately 90–95% of cases with manifested diabetes⁽¹⁾. Due to the detrimental consequences and diabetes-associated disorders (e.g. retinopathy, neuropathy and CVD), it is necessary to use every available tool to prevent the onset as well the progression of the disease. Again, type 2 diabetes is of prime importance, as its pathogenesis can be prevented by lifestyle modifications. Increased physical activity and improved nutritional habits in the form of healthy balanced diets are of particular importance in the deceleration of type 2 diabetes manifestations. In 2003, the American Diabetes Association classified an intermediate group of individuals who did not meet the criteria for symptomatic diabetes with respect to their glucose levels. However, due to impaired fasting glucose (FG) levels (values of fasting

plasma glucose in the range of 5.6–6.9 mmol/l) and impaired glucose tolerance (2 h values of plasma glucose in the range of 7.8–11 mmol/l following an oral glucose tolerance test) or permanently increased glycosylated Hb levels (5.7–6.4% or 38.7–46.4 mmol/mol), these individuals are considered to be at an increased risk of type 2 diabetes and termed pre-diabetics. Impaired FG levels and impaired glucose tolerance are usually associated with obesity (especially increased abdominal and visceral fat mass), dyslipidaemia, increased plasma TAG levels, decreased plasma HDL-cholesterol (HDL-C) levels and hypertension, all representing established type 2 diabetes risk factors⁽²⁾. Numerous long-term intervention studies have shown that lifestyle improvements exert beneficial effects on the onset and progression of type 2 diabetes. Thus, both weight reduction and physical exercise have been shown to be significantly associated with a decreased incidence of diabetes^(3,4).

Abbreviations: DBP, diastolic blood pressure; FG, fasting glucose; FI, fasting insulin; HDL-C, HDL-cholesterol; HF, high fat; LC, low carbohydrate; LF, low fat; MD, mean differences; RCT, randomised controlled trials; SBP, systolic blood pressure; TC, total cholesterol; TEC, total energy content.

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To accomplish the objective of weight reduction, the American Diabetes Association recommended energy-reduced dietary protocols without giving any preference to macronutrient composition. According to its position statement, both a low-carbohydrate (LC) regimen and a low-fat (LF) regimen are considered to be effective means for weight management⁽⁵⁾. In a meta-analysis published in 2009, Kodama *et al.*⁽⁶⁾ investigated the short-term effects of LF *v.* LC diet consumption in patients with type 2 diabetes. They could observe significantly more pronounced changes in fasting insulin (FI), TAG and HDL-C levels, all in favour of the LC dietary protocol. With respect to their macronutrient distribution, it seems legitimate to put high-fat (HF) diets on a level with LC diets, assuming that a reduced carbohydrate content will most probably be accompanied by an increased amount of fat in the total energy content (TEC). The aim of the present systematic review was to examine the long-term effects (≥ 12 months) of HF *v.* LF diet consumption on the parameters of glycaemic control in both pre-diabetics and patients with established type 2 diabetes. Furthermore, cardiovascular risk factors (e.g. TAG, LDL-cholesterol and HDL-C) were included as outcome parameters. As has been described previously, a HF diet was defined to provide $>30\%$ of TEC in the form of fat, whereas $\leq 30\%$ of TEC was taken up in the form of fat in the LF counterparts⁽⁷⁾. In this context, the data reported by Kodama *et al.*⁽⁶⁾ would argue against the equivalent usefulness of LF (= high-carbohydrate) and LC (= HF) diets emphasising the value of the latter. However, the validity of this conclusion has to be substantiated by the analysis of long-term studies including the objectives of primary prevention, i.e. investigating the effects of different dietary protocols on risk parameters in pre-diabetic individuals.

Methods

Data sources and searches

Literature search restricted to randomised controlled trials (RCT) without any restrictions on language and calendar date was carried out using the electronic databases MEDLINE (until November 2013), Embase (until November 2013) and the Cochrane Trial Register (until November 2013) with the following search terms: low-fat diet; high-fat diet; low-carbohydrate diet; high-carbohydrate diet. Full details of the electronic search strategy are given in the online supplementary material. Furthermore, the reference lists from the retrieved articles were checked to search for further relevant studies. This systematic review was planned, conducted and reported in adherence to the standards of quality for reporting meta-analyses⁽⁸⁾. Literature search as well as article abstraction was conducted independently by both the authors, with disagreements being resolved by consensus.

Study selection

Studies were included in the meta-analysis if they met all the following criteria: (1) randomised controlled design; (2) minimum intervention period with a follow-up period of

12 months; (3) comparison of a HF dietary intervention ($>30\%$ of TEC) with a LF dietary intervention ($\leq 30\%$ of TEC), stated as the goal of intervention; (4) age of the subjects ≥ 18 years; (5) enrolment of subjects with either impaired FG levels (≥ 5.6 mmol/l) or insulin resistance⁽⁹⁾ or type 2 diabetes⁽²⁾; (6) assessment of the 'outcome of interest' markers: body weight, total cholesterol (TC), LDL-cholesterol, HDL-C, TC:HDL-C ratio, TAG, systolic and diastolic blood pressure (SBP/DBP), C-reactive protein, FI, FG, glycosylated Hb, and adiponectin; (7) report of post-intervention mean or mean of values recorded at two time points with standard deviation (or basic data to calculate these parameters). If data of ongoing studies were published as updates, results of only those of the longest duration were included. Studies that enrolled patients with type 1 diabetes mellitus were excluded.

Data extraction and quality assessment – overall quality of evidence

The focus of this systematic review was the examination of the effects of LF *v.* HF diet consumption on the values of glycaemic control and cardiovascular risk factors in individuals at a high risk of type 2 diabetes or with manifested type 2 diabetes. The following types of dietary interventions were evaluated: in the primary analysis, LF diets were defined as those providing $\leq 30\%$ of TEC in the form of fat, approximately 15% of TEC from protein and approximately 55% of TEC from carbohydrates and HF diets were defined as those providing $>30\%$ of TEC as fat. HF diets were further classified according to alternative macronutrient composition as follows:

- (1) usual diet (total fat content $>30\%$ of TEC and SFA content $>10\%$ of TEC);
- (2) LC diet (<50 g carbohydrates/daily);
- (3) high-monounsaturated fat, MUFA diet (total fat content $>30\%$ of TEC and total MUFA content $>12\%$ of TEC).

The risk of bias assessment tool provided by the Cochrane Collaboration was applied specifying the following bias domains: selection bias (random sequence generation and allocation concealment); performance/detection bias (blinding of participants and personnel/blinding of outcome assessment); attrition bias (incomplete data outcome); reporting bias (selective reporting); other bias (see online supplementary Fig. S1)⁽¹⁰⁾.

The following data were extracted for each study: the first author's last name; year of publication; study duration; sex distribution and age; BMI; percentage of diabetics; sample size; outcomes; post-intervention mean values or differences in the mean of values recorded at two time points with the corresponding standard deviation. The quality of evidence was assessed according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines⁽¹¹⁾.

Data synthesis and analysis

For each outcome measure of interest, a meta-analysis was carried out to determine the pooled effect of the intervention

in terms of weighted mean differences (MD) between the post-intervention (or differences in means) values of the HF group and those of the LF group. Combining both the post-intervention values and differences in means in one meta-analysis is a legitimate method described by the Cochrane Collaboration⁽¹²⁾. All data were analysed using the Review Manager 5.1 software, provided by the Cochrane Collaboration (<http://ims.cochrane.org/revman>). The random-effects model was used to estimate MD with 95% CI. Forest plots were generated to illustrate the study-specific effect sizes along with 95% CI. Heterogeneity between the trial results was tested with a standard χ^2 test. The I^2 parameter was used to quantify any inconsistency:

$$I^2 = ((Q - df)/Q) \times 100\%,$$

where Q is the χ^2 statistic. A cut-off point $\geq 50\%$ was chosen for I^2 to indicate substantial heterogeneity⁽¹²⁾. To evaluate substantial heterogeneity, several *post hoc* univariate random-effects meta-regressions were performed to examine the association between total fat, SFA, MUFA, PUFA, carbohydrate, protein, dietary cholesterol and fibre intake as independent variables and changes in FG, FI, TC and HDL-C levels (where substantial heterogeneity could be detected) as dependent variables. Furthermore, BMI, age, sex and study duration were used as covariates. The P values for differences in effects between the covariates were obtained using the *metareg* function of Stata 12.0 (Stata-Corporation). Two-sided P values < 0.05 were considered to be statistically significant. To increase the precision of the estimates of macronutrient intake (total fat, SFA, MUFA, PUFA, fibre, cholesterol, carbohydrate and protein (all in percentage of TEC)), data from FFQ, 24 h dietary recalls or 3–7 d dietary protocols (if available) were used instead of the theoretical macronutrient composition values of dietary intervention protocols. Funnel plots were sketched to indicate potential publication bias (e.g. the tendency for studies yielding statistically significant results to be more likely to be submitted and accepted for publication). To determine the presence of publication bias, the symmetry of the funnel plots in which MD were plotted against their corresponding standard errors was assessed. A primary analysis of all studies oriented towards the definition of HF and LF diets was carried out, followed by a subanalysis of the specific kind of dietary intervention as described in the selected studies. In addition, a sensitivity analysis including only subjects with type 2 diabetes as well as a sensitivity analysis to determine the risk of bias of the trials^(13,14) was conducted.

Among the studies included in the review, two studies^(15,16) used two types of LF diets, and these diets were combined into one group as described in the Cochrane Handbook⁽¹²⁾. In the study carried out by Shai *et al.*⁽¹⁷⁾, the LF and LC branches were extracted for meta-analysis, while the MUFA group was discarded and re-analysed in the sensitivity analyses, as duplicate application of the LF dietary intervention data would not be legitimate. Data extraction was conducted independently by both the authors, with disagreements being resolved by consensus. Data processing for this review required the input of the mean and standard deviation

of post-intervention values or differences in means. In case of missing data, the authors of the original article were asked for additional information and, if provided, the raw data were used for computations^(16,17).

Results

Literature search

A total of fourteen studies extracted from 16 608 articles met the inclusion criteria and were analysed in the systematic review^(15–30). Detailed steps of the article selection process used for the present meta-analysis are shown as a flow chart in Fig. 1. In accordance with the overall inclusion criteria, five studies were excluded due to inconsistencies in the mean FG levels of the study populations (≥ 5.6 mmol/l) and the corresponding standard deviations, thereby increasing the potential for selection bias (Fig. 1)^(31–35).

Study and participant characteristics

All studies included in this systematic review were RCT with a duration ranging between 12 months and 6 years, published between 1978 and 2012, and enrolling a total of 2003 participants. All studies compared a HF regimen defined as a LC diet (six studies), a control/HF diet (four studies) or a MUFA-rich protocol (four studies) with a LF regimen. The reported BMI was > 25 kg/m² in all the studies and the mean age of the participants varied between 52 and 62 years. The reported drop-out rates were 25% for the HF group and 23% for the LF group. The general study characteristics are given in Table 1. Results obtained using the risk of bias assessment tool are summarised in online supplementary Fig. S1.

Outcomes

The pooled estimates of weighted MD for the effects of HF diet consumption compared with those of LF diet consumption on body weight, blood lipids and parameters of glycaemic control are summarised in Table 2. Changes in body weight (see online supplementary Fig. S2), TC levels (see online supplementary Fig. S3), LDL-cholesterol levels (see online supplementary Fig. S4), SBP (see online supplementary Fig. S8), FI levels (see online supplementary Fig. S9), FG levels (see online supplementary Fig. S10), glycosylated Hb levels (see online supplementary Fig. S11), TC:HDL-C ratio and C-reactive protein levels in subjects following a HF diet were not significantly different from those in subjects following a LF diet.

The HF dietary protocols were found to lead to a significantly more pronounced decrease in TAG levels when compared with the LF dietary protocols (MD -0.19 mmol/l, 95% CI -0.23 , -0.14 , $P < 0.001$; $I^2 = 0\%$, $P = 0.58$). The subgroup analyses revealed that the decrease in TAG levels was significant in subjects following a MUFA-rich diet than in those following a LF diet (MD -0.20 mmol/l, 95% CI -0.25 , -0.15 , $P < 0.001$; $I^2 = 13\%$, $P = 0.33$; see online supplementary Fig. S6).

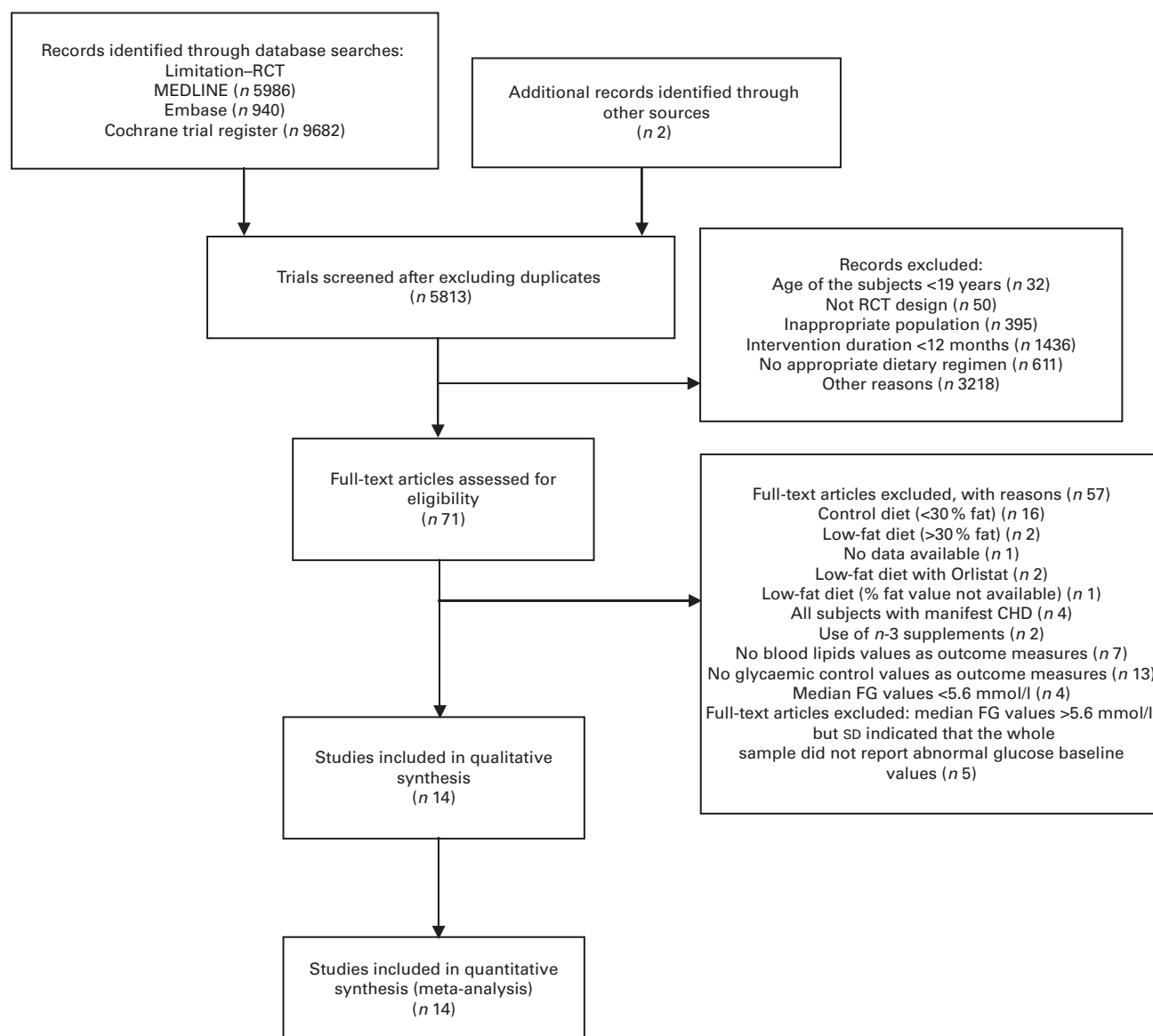


Fig. 1. Flow chart for the systematic review article selection process. RCT, randomised controlled trials; FG, fasting glucose.

HDL-C (see online supplementary Fig. S5; MD 0.05 mmol/l, 95% CI 0.01, 0.08, $P=0.01$; $I^2=57\%$, $P=0.01$) and adiponectin (MD 1.10 $\mu\text{g/ml}$, 95% CI 0.87, 1.33, $P<0.001$) levels were significantly more increased by the HF dietary interventions than by the LF dietary interventions. Subgroup analyses yielded non-significant results.

With respect to blood pressure values, reductions in DBP were significantly more explicit in subjects adhering to a HF diet than in those adhering to a LF diet (MD -1.30 mmHg, 95% CI -1.73 , -0.87 , $P<0.001$; $I^2=0\%$, $P=0.60$; see online supplementary Fig. S7). Comparable results for both DBP (MD -1.38 mmHg, 95% CI -1.82 , -0.95 , $P<0.001$; $I^2=0\%$, $P=0.79$) and SBP (MD -1.48 mmHg, 95% CI -2.01 , -0.96 , $P<0.001$; $I^2=0\%$, $P=0.45$) were obtained when comparing high-MUFA groups with LF groups in subgroup analyses.

With regard to biomarkers of glycaemic control, changes in FI levels did not differ between the HF and LF groups. However, *post hoc* analysis of subgroups revealed that FI values were significantly more increased in subjects adhering to a usual/HF diet than in those adhering to a LF diet (MD 7.79 pmol/l, 95% CI 3.24, 12.33, $P<0.001$; $I^2=0\%$, $P=0.95$).

Sensitivity/subgroup analyses

To investigate the effects of HF *v.* LF diet consumption in patients with manifested type 2 diabetes, a sensitivity analysis was carried out by excluding all RCT enrolling only subjects at a risk of type 2 diabetes (i.e. individuals with impaired FG levels or insulin resistance). A total of eleven studies remained for secondary analyses^(15,17,19–23,26,29,30,36). The results turned out to be not significantly different from those of the comprehensive

Table 1. Characteristics of the randomised controlled intervention trials included in the present meta-analysis

Reference	Participants, BMI (kg/m ²), diabetics (%)	Age (years), female (%)	Diagnostic criteria for T2D, IFG and IR	Duration (years)	Dietary intervention	Dietary protocol: fat, protein, CH	Macronutrient intake at the end of the follow-up period: fat, protein, CH	Energy restricted		Dietary assessment	Dropout rate (%)	Hypoglycaemic agents (%)
								kcal	kJ			
Brehm <i>et al.</i> ⁽¹⁹⁾	95	56.5	HbA1c*: 6.5–9 % (36.5–75 mmol/mol)	1	HF (MUFA) v.	40 %, 15 %, 45 %, 20 % MUFA (olive oil and canola oil)	38 %, 16 %, 46 %	– 200 to – 300	– 837 to – 1255	3 d food record	31	In thirty-two subjects, drug usage was tracked
	35.9 100	64			LF	25 %, 15 %, 60 %	28 %, 18 %, 54 %	– 200 to – 300	– 837 to – 1255		16	
Davis <i>et al.</i> ⁽²⁰⁾	105	53.5	HbA1c: 6–11 % (42–97 mmol/mol)	1	HF (LC) v.	20–25 g/d CH for 2 weeks, increase 5 g/week (CH)	44 %, 23 %, 33 %	No	No	24 h recall	19	78 Metformin 44 Sulphonylureas 35 Insulin
	36 100	75			LF	25 %	31 %, 19 %, 50 %	No	No		19	86 Metformin 52 Sulphonylureas 24 Insulin ND
Elhayany <i>et al.</i> ⁽¹⁵⁾	179	56.3	HbA1c: 7–10 % (53–86 mmol/mol)	1	HF (MUFA) v.	45 %, 20 %, 35 %, 23 % MUFA	Not reported	1700	7113	24 h recall and FFQ	28	ND
	31.4 100	48			LF†	30 %, 20 %, 50 %	Not reported	1700	7113		32	
Esposito <i>et al.</i> ⁽²¹⁾	215	52.2	HbA1c: > 7 % (> 53 mmol/mol)	4	HF (MUFA) v.	> 30 %, 15 %, < 50 %, 30–50 g/d olive oil	40 %, 17 %, 43 %	1500 women and 1800 men	6276 women and 7531 men	Diet diaries	9	Inclusion criteria: T2D subjects never been treated with antihyperglycaemic drugs
	29.6 100	51			LF	< 30 %, 15 %, 55 %	29 %, 18 %, 53 %	1500 women and 1800 men	6276 women and 7531 men		9	
Guldbrand <i>et al.</i> ⁽²²⁾	61	62	Diagnosis of T2D treated with diet with or without additional glucose-lowering medication, incretin-based therapy or insulin	2	HF (LC) v.	50 %, 30 %, 20 %	44 %, 24 %, 31 %	1600 women and 1800 men	6694 women and 7531 men	3 d diet record	10	Metformin
	32.7 100	56			LF	< 30 %, 10–15 %, 60 %	31 %, 20 %, 47 %	1600 women and 1800 men	6694 women and 7531 men		13	Insulin Glibenclamide
Hockaday <i>et al.</i> ⁽²³⁾	93 ND 100	51.5 56	After 1 h 50 g glucose tolerance test: 10.6 mmol/l	1	HF (control) v. LF	40 %, 20 %, 40 % 26 %, 20 %, 54 %	Not reported Not reported	1500 1500	6276 6276	ND	ND	ND
	759 ND 29	ND 100	IFG: 5.6–6.9 mmol/l Diabetes history or FG: > 6.9 mmol/l	6	HF (control) v. LF	> 30 % < 20 %, < 7 % (SFA)	37 %, 17 %, 46 % 29 %, 17 %, 54 %	No No	No No	FFQ	ND	Self-reported treatment of diabetes, 80 % were confirmed to use an anti-diabetic medication
Iqbal <i>et al.</i> ⁽²⁶⁾	68	60	Diabetes defined as a pre-existing clinical diagnosis or use of insulin or oral anti-diabetic medications	2	HF (LC) v.	30 g/d CH	34 %, 17 %, 49 %	No	No	Self-reported dietary intake	54	57 Sulphonylureas 61 Metformin 9 Thiazolidinedione 23 Insulin
	37.5 100	11			LF	< 30 %	34 %, 18 %, 48 %	– 500	– 2092		40	43 Sulphonylureas 53 Metformin 11 Thiazolidinedione 30 Insulin

High-fat v. low-fat diets

2051

Table 1. Continued

Reference	Participants, BMI (kg/m ²), diabetics (%)	Age (years), female (%)	Diagnostic criteria for T2D, IFG and IR	Duration (years)	Dietary intervention	Dietary protocol: fat, protein, CH	Macronutrient intake at the end of the follow-up period: fat, protein, CH	Energy restricted		Dietary assessment	Dropout rate (%)	Hypoglycaemic agents (%)
								kcal	kJ			
Ley <i>et al.</i> ⁽²⁷⁾	103	52.3	IGT: 2 h blood glucose 7–11 mmol/l;	5	HF (control) v.	>30 %	35 %, 18 %, 47 %	No	No	3 food diary	ND	ND
Swinburn <i>et al.</i> ⁽²⁸⁾	29.2	26	WHO 1985		LF	<30 %	26 %, 19 %, 55 %	No	No			
McAuley <i>et al.</i> ⁽¹⁸⁾	48	ND	Reduced predicted insulin sensitivity (insulin sensitivity score $\leq 6.3 \text{ M} \times \text{mL per l}^{(9)}$)	1	HF (LC) v.	20 g/d CH for 2 weeks, 50 g/d for 8 weeks	41 %, 21 %, 33 %	No	No	3 food diary	23	ND
	ND	100			LF	<30 %, 15 %, 55 %	29 %, 22 %, 45 %	No	No		25	
Milne <i>et al.</i> ⁽²⁹⁾	43	59.5	Duration of diabetes: 5–5.6 years;	1.5	HF (control) v.	36 %, 19 %, 45 %	34 %, 20 %, 46 %	– 500	– 2092	24 h recall	ND	50
	29.5	53	HbA1c: 8.7–9.8 % (71.7–83.8 mmol/mol)		LF	30 %, 15 %, 55 %	32 %, 21 %, 47 %	– 500	– 2092			57
	100											
Shai <i>et al.</i> ⁽¹⁷⁾	19	ND	According to ADA 1997	2	HF (LC) v.	20 g/d CH; increase to max 120 g/d	39 %, 21 %, 40 % (whole sample size)	No	No	FFQ	ND	70 Oral agents 11 Insulin
	ND	ND			LF	<30 %	30 %, 19 %, 51 % (whole sample size)	1500 women and 1800 men	6276 women and 7531 men			50 Oral agents 17 Insulin
	100	ND										
Stern <i>et al.</i> ⁽³⁰⁾	54	ND	Diabetes defined as a pre-existing clinical diagnosis or use of insulin or oral anti-diabetic medications	1	HF (LC) v.	<30 g/d CH	57 %, 20 %, 33 %	No	No	Dietary recall data	31	22 Sulphonylureas 44 Metformin 5 PPAR agonist 17 Insulin
	ND	ND			LF	<30 %	34 %, 16 %, 50 %	– 500	– 2092		37	43 Sulphonylureas 32 Metformin 5 PPAR agonist 14 Insulin
	100	ND										
Wolever <i>et al.</i> ⁽¹⁶⁾	156	59.86	FG: >7 mmol/l or >11 mmol/l after 2 h OGTT	1	HF (MUFA) v.	>35 %, ↑ fat intake by about 10 % replacing CH (by nuts, olive oil and canola oil)	Not reported	– 500‡	– 2092‡	3 d food record	19	Exclusion criteria: use of insulin or any hypoglycaemic or antihyperglycaemic medication
	30.9	54			LF†	<30 %	Not reported	– 500‡	– 2092‡		20	
	100											

T2D, type 2 diabetes; IFG, impaired fasting glucose; IR, insulin resistance; CH, carbohydrates; HbA1c, glycosylated Hb; HF, high fat; LF, low fat; LC, low carbohydrate; ND, not determined; IGT, impaired glucose tolerance; ADA, American Diabetes Association; PPAR agonist, PPAR-γ agonist; OGTT, oral glucose tolerance test.

* To convert HbA1c to mmol/mol: $10.93 \times \text{HbA1c unit (\%)} - 23.50$.

† Two kinds of LF diets (high-glycaemic index and low-glycaemic index; 10 and 30 % of total energy consumption).

‡ In case subjects wished to lose weight.

Table 2. Pooled estimates of effect size (95% CI) expressed as weighted mean difference (MD) for the effects of high-fat *v.* low-fat diet consumption on cardiovascular and metabolic risk factors

Outcome parameters	No. of studies	No. of participants	MD	95% CI	P	Inconsistency I^2 (%)	Quality of evidence (GRADE)
BW (kg)	11	1172	-0.11	-1.14, 0.91	0.83	0	Moderate*
BW (kg)†	8	928	-0.47	-1.85, 0.92	0.51	0	
TC (mmol/l)	11	1148	0.07	-0.10, 0.23	0.42	67	Moderate*‡
TC (mmol/l)†	9	997	0.08	-0.11, 0.27	0.41	73	
LDL-C (mmol/l)	9	836	0.05	-0.10, 0.20	0.53	49	Moderate*
LDL-C (mmol/l)†	7	685	0.04	-0.14, 0.23	0.64	60	
HDL-C (mmol/l)	11	1290	0.05	0.01, 0.08	0.01	57	Moderate*
HDL-C (mmol/l)†	9	1139	0.04	0.00, 0.08	0.03	65	
TAG (mmol/l)	12	1384	-0.19	-0.23, -0.14	<0.00001	0	Moderate*
TAG (mmol/l)†	10	1233	-0.18	-0.24, -0.13	<0.00001	4	
TC:HDL-C	2	240	0.06	-0.38, 0.50	0.80	34	Very low*
CRP (mg/l)	1	138	-1.31	-2.91, 0.29	0.11	/	Very low*
DBP (mmHg)	8	827	-1.30	-1.73, -0.87	<0.00001	0	Low*§
DBP (mmHg)†	6	676	-1.35	-1.79, -0.92	<0.00001	0	
SBP (mmHg)	7	695	0.59	-2.18, 3.36	0.68	40	Low*
SBP (mmHg)†	5	310	-1.35	0.35, 2.35	0.008	3	
FG (mmol/l)	11	1753	-0.18	-0.52, 0.15	0.28	82	Moderate*‡
FG (mmol/l)†	9	1062	-0.41	-0.74, -0.08	0.01	56	
FI (pmol/l)	10	1718	2.93	-3.30, 9.15	0.36	71	Moderate*‡
FI (pmol/l)†	8	994	0.61	-6.66, 7.89	0.87	58	
HbA1c (%)†	10	981	-0.17	-0.39, 0.06	0.14	46	Moderate*
HbA1c (mmol/mol)†	10	981	-1.055	-2.418, 0.372	0.14	46	Moderate*
Adiponectin (µg/ml)†	1	215	1.10	0.87, 1.33	<0.00001		Very low*§

GRADE, Grading of Recommendations Assessment, Development and Evaluation; BW, body weight; TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; CRP, C-reactive protein; DBP, diastolic blood pressure; SBP, systolic blood pressure; FG, fasting glucose; FI, fasting insulin; HbA1c, glycosylated Hb.

* Risk of bias in many studies.

† Sensitivity analysis: including studies with only subjects with type 2 diabetes.

‡ Heterogeneity was observed, but could not be explained.

§ Large study effects were observed; if studies excluded from the analysis, the MD became non-significant.

meta-analyses except for one parameter: detrimental effects on FI values were no longer valid when comparing usual *v.* LF protocols in the respective subgroup analysis.

Moreover, additional parameters were specifically altered by HF diet consumption in patients with type 2 diabetes. Both SBP (MD -1.35 mmHg, 95% CI -2.35, -0.35, $P=0.008$; $I^2=3\%$, $P=0.39$) and FG (MD -0.41 mmol/l, 95% CI -0.74, -0.08, $P=0.01$; $I^2=56\%$, $P=0.02$) levels were significantly more decreased in subjects following a HF diet than in those following a LF diet. In the Women's Health Initiative as well as the Workforce Diabetes Survey trials^(24,25,27,28), the HF dietary intervention group was indicated as a control group receiving only 'usual care'. By implication, one might expect a bias due to the fact that the intervention (i.e. the LF) group received more attention from the investigators, e.g. in form of education, personal goal settings and regular meeting. However, the results of the primary analysis were not affected by the exclusion of these trials.

Subgroup analyses for change scores, hypoenergetic diets, *ad libitum* diets, study duration (long term and short term), high adherence and low risk of bias were carried out (see online supplementary Tables S1–S7). The majority of the results of the main analysis could be confirmed. Inclusion of only *ad libitum* diets significantly reduced heterogeneity. Information on the prescription of hypoglycaemic agents was extracted, and it is reported in Table 1. However, due to inconsistent reporting, the data did not allow carrying out further analyses.

Publication bias

The funnel plots (with respect to effect size changes for biomarkers of cardiovascular risk and glycaemic control in response to LF diet consumption) revealed very little asymmetry, suggesting low evidence of publication bias (see online supplementary Figs. S12–S21).

Heterogeneity

Substantial heterogeneity was found with respect to TC ($I^2=67$; 73%), HDL-C ($I^2=57$; 65%), FG ($I^2=82$; 56%) and FI ($I^2=71$; 58%) levels in both primary and secondary analyses (Table 2). It was assumed that substantial heterogeneity might be explained by non-uniform study characteristics of the HF groups such as variations in post-intervention macronutrient intake. To gain insight into these potential correlations, a random-effects meta-regression was performed to examine the associations between LF and HF group parameters and changes in TC, HDL-C, FG, and FI levels. Studies with a higher percentage of energy from carbohydrates were associated with slightly lower differences in TC levels between the two dietary intervention groups (0.026 mmol/l lower TC for every 1% increase in energy from carbohydrates; 95% CI 0.050, 0.002; $P=0.036$; Fig. 2(a)), those with a higher percentage of energy from fat were associated with slightly higher differences in FI levels between the two dietary intervention groups (2.67 pmol/l higher FI for every 1% increase in

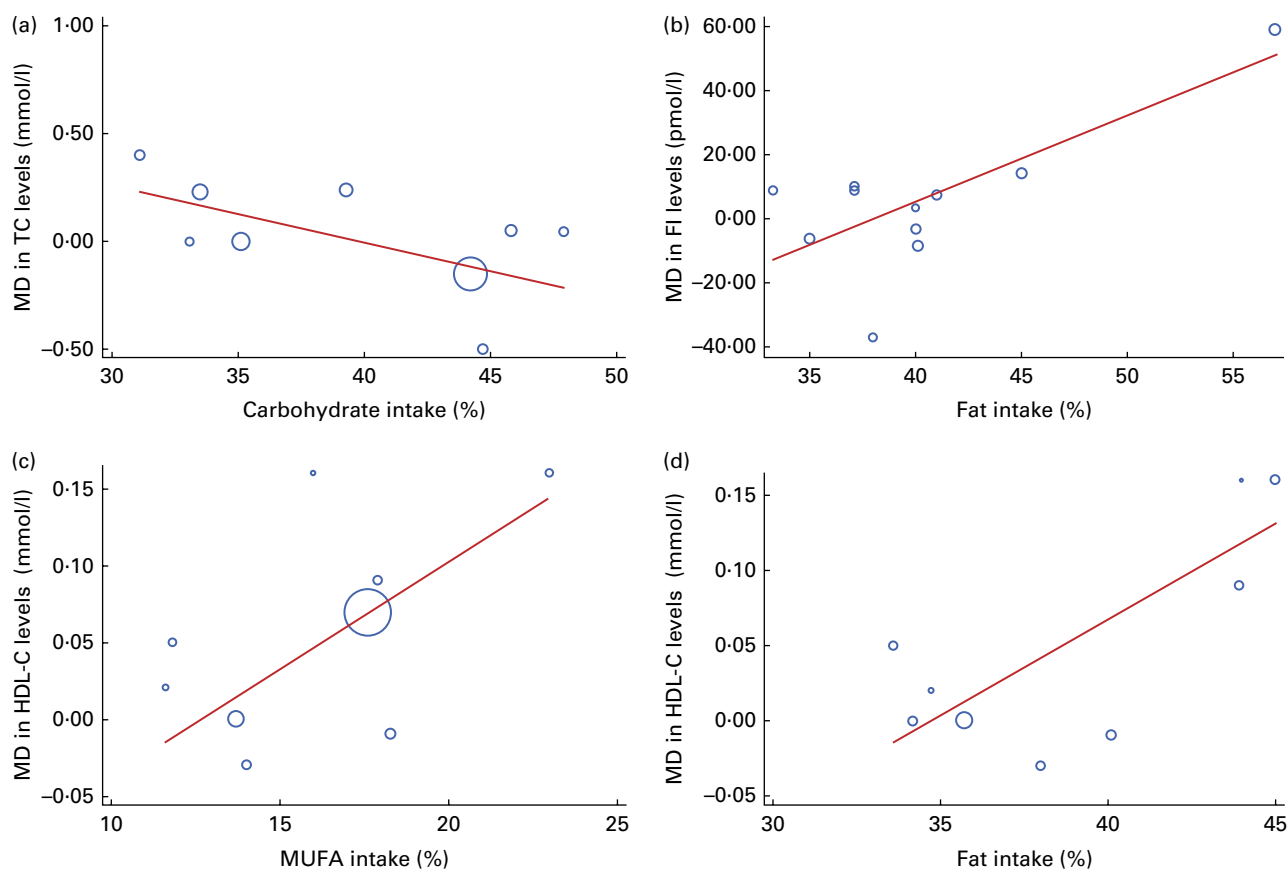


Fig. 2. Bubble plots showing the dose–response relationship between (a) carbohydrate intake and changes in total cholesterol (TC) levels ($P=0.036$), (b) fat intake ($P=0.006$) and changes in fasting insulin (FI) levels, (c) MUFA intake and changes in HDL-cholesterol (HDL-C) levels ($P=0.015$), and (d) total fat intake and changes in HDL-C levels ($P=0.005$). Macronutrient intake is given as the percentage of total energy consumption. The fitted regression lines together with the bubbles represent the estimates from each study, sized according to the precision of each estimate as calculated in the fitted random-effects meta-regression. MD, mean difference. (A colour version of this figure can be found online at <http://www.journals.cambridge.org/bjn>).

energy from fat; 95 % CI 0.959, 4.38; $P=0.006$; Fig. 2(b)), and those with a higher percentage of energy from MUFA and total fat were associated with slightly higher differences in HDL-C levels between the two dietary intervention groups (0.014, 0.012 mmol/l higher HDL-C for every 1 % increase in energy from MUFA and total fat; 95 % CI 0.003, 0.024; $P=0.015$; 95 % CI 0.004, 0.020, $P=0.005$; Fig. 2(c) and (d)). No such correlations could be detected for the other parameters under investigation.

Overall quality of evidence

The overall quality of evidence rated according to the GRADE guidelines ranged from very low to moderate (Table 2). Moderate-quality evidence was found concerning the significant reduction in TAG levels as well as the significant increase in HDL-C levels in subjects following a HF diet. Similarly, moderate-quality evidence could be observed with respect to the non-significant effects of HF regimens on TC and LDL-cholesterol levels. Moderate-quality evidence was observed for the effects of HF diet consumption on the parameters of glycaemic control as outcomes as well. For the remaining outcome parameters (blood pressure, C-reactive protein and adiponectin), low-to-very-low-quality evidence was observed.

Discussion

Type 2 diabetes mellitus is one of the most pressing non-communicable chronic diseases with an estimated prevalence of approximately 235 million affected cases by the year 2030. In the pathogenesis of its manifestation, type 2 diabetes is preceded by a most often undetected phase of pre-diabetes with impaired glucose metabolism, which, however, is susceptible to the benefits of lifestyle changes⁽²⁾. Various biomarkers have been shown to be predictors of the detrimental consequences of diabetes-associated disorders resulting from micro- and macroangiopathies. In the present systematic review and meta-analysis, long-term intervention studies enrolling either patients with type 2 diabetes or subjects with pre-diabetes and comparing a HF dietary regimen with a LF dietary regimen were analysed. The primary analysis revealed a favourable effect of HF diet consumption with respect to TAG levels (decrease), DBP (decrease), HDL-C levels (increase) as well as adiponectin levels (increase), although the last-mentioned biomarker was measured in only one study including 215 diabetic volunteers⁽²¹⁾. By contrast, an advantage of a LF protocol could be observed when compared with its usual/HF counterpart with respect to FI values in the subgroup analyses. However, this benefit

was no longer present following sensitivity analysis including studies with only patients with type 2 diabetes. Thus, a major finding of this meta-analysis is that HF diets exert beneficial effects – when compared with LF diets – on a number of biomarkers considered to be predictors of diabetes-associated complications.

CHD is a highly prevalent manifestation of microangiopathies associated with diabetes and has been reported to have caused 12% of all premature deaths worldwide in 2004⁽³⁷⁾. With respect to HDL-C levels, a previous epidemiological study has shown that an increase of approximately 0.025 mmol/l is associated with a decrease in CVD risk of 2% in men and 3% in women⁽³⁸⁾. In the present meta-analysis, HF protocols were found to result in an average higher increase of 0.05 mmol/l in plasma HDL-C levels when compared with LF protocols, indicating a greater risk reduction in CHD by 3.75–5.5%. Augmented plasma levels of TAG are considered to be univariate predictors of CVD as well⁽³⁹⁾. An increase of 1 mmol/l in plasma levels is associated with a 2-fold increase in the relative risk of CHD⁽⁴⁰⁾. On combining these data with the results of the present meta-analysis, it was found that the decline in TAG levels following HF diet consumption would be associated with a reduced CHD risk of approximately 10%.

Increased blood pressure intensifies the risk of stroke and CHD in patients with type 2 diabetes and it could be shown that even minor reductions in blood pressure will reduce the incidence of CVD. Improvements in mean arterial pressure (–3 mmHg) were found to be correlated with a reduction in the risk of CVD (5–10%), stroke (8–15%) and all-cause mortality (5%)⁽⁴¹⁾. Moreover, a single reduction of SBP (–3%) was found to be associated with a decreased risk of fatal myocardial infarctions of approximately 8%⁽⁴²⁾. The present data suggest a beneficial effect of HF diet consumption on DBP in patients with type 2 diabetes and subjects with pre-diabetes, while the sensitivity analysis including only patients with manifested diabetes revealed a beneficial effect on SBP as well (when compared with LF diet consumption).

A significant decrease in FG values (–0.41 mmol/l) was found in patients with type 2 diabetes subjected to a HF diet challenge. According to the Asian Pacific Study, attenuations in FG levels of 180 mg/l are correlated with a 23% diminished risk of CVD⁽⁴³⁾. Moreover, the authors of the United Kingdom Prevention Study considered hyperglycaemia to be a more relevant predictor of coronary events in the course of type 2 diabetes when compared with increased insulin levels⁽⁴⁴⁾. This hypothesis is supported by the findings of the Emerging Risk Factors Collaboration Study reporting a significant correlation between FG levels >5.6 mmol/l and increased mortality risk⁽⁴⁵⁾.

Among the studies included in the present meta-analysis, one study reported that HF diets increased plasma adiponectin levels. Epidemiological data as well as experimental studies have indicated that decreased plasma adiponectin values are associated with a pronounced risk of insulin resistance and manifestation of type 2 diabetes⁽⁴⁶⁾.

When compared with the findings of the present meta-analysis, similar data have been reported in meta-analyses investigating short-term RCT. Thus, Kodama *et al.*⁽⁶⁾ observed

significant increases in TAG levels as well as decreases in HDL-C levels in patients with type 2 diabetes subjected to a LF diet challenge than in those of patients subjected to a HF diet challenge. Weighing moderate-fat diets against a regimen with LF intake, Cao *et al.*⁽⁴⁷⁾ found significant changes in TAG levels, HDL-C levels and TC:HDL-C ratio in favour of the moderate-fat protocol. Following a meta-regression analysis, the authors concluded that at least the changes observed in HDL-C levels were correlated with total fat as well as unsaturated fat intake, suggesting that a reduction in carbohydrate intake with simultaneous increase in unsaturated fat (MUFA and PUFA) intake would exert a beneficial effect on plasma lipoproteins. The adherence of individuals assigned to a LC dietary intervention might change over time. Usually, there is good adherence in the short term, but it gets poorer in the long term⁽⁴⁸⁾, which might explain the benefits of LC diet consumption observed by Kodama *et al.*⁽⁶⁾. In the present meta-analysis, macronutrient intakes in the included trials were found to be altered sometimes at the end of the follow-up period. However, in most studies, the dietary protocol at the end of the follow-up period was still distinguishable with respect to HF or LF intakes (Table 1). However, it should be noted that the HF and LF diets had roughly the same dietary macronutrient composition at the end of the follow-up period in the studies carried out by Iqbal *et al.*⁽²⁶⁾ and Milne *et al.*⁽²⁹⁾.

In this meta-analysis, a substantial heterogeneity ($I^2 > 50\%$) was found for TC, HDL-C, FG and FI levels (Table 2). Following meta-regressions to examine the associations between HF and LF diet consumption and changes in the outcome parameters, a statistically significant relationship was detected between carbohydrate intake and decreases in TC levels (probably caused by a reduction in SFA intake)⁽⁷⁾, fat intake and increases in FI levels, and total fat and MUFA intake and increases in HDL-C levels. This is in accordance with the findings from other meta-analyses^(49–54). By comparing the results of four recent meta-analyses, Pagoto & Appelhans⁽⁵⁵⁾ suggested that investigations dealing with different dietary macronutrient approaches show only small differences between the diets. This statement could be confirmed by the results of the present meta-analysis.

Limitations of the present systematic review

The data of the present meta-analysis refer only to values obtained after an overnight fast and, as such, only represent part of the glycaemic control data; for example, there are no postprandial or post-glucose challenge data. Moreover, the present systematic review did not consider unpublished results, and it cannot be excluded that these results may have had at least a moderate impact on the effect size estimates. Examination of funnel plots revealed very little asymmetry, suggesting that the evidence for publication bias is of low quality. A major limitation of nutritional intervention trials is the heterogeneity of various aspects and characteristics of the study protocols. Therefore, it is not surprising that the RCT included in the present meta-analysis varied regarding the type(s) of diets used (energy restriction, isoenergetic), definitions of LF and HF diets, study population (i.e. age,

BMI, type 2 diabetics, abnormal glucose metabolism), intervention time and nutritional assessment as well as long-term follow-up periods (1–6 years.). Following sensitivity analysis including only studies enrolling patients with type 2 diabetes, the beneficial effects of HF diet consumption on TAG levels, HDL-C levels and DBP were found to remain the same as those observed in the conclusive analyses. Moreover, HF protocols were found to exert a more favourable effect than their LF counterparts with respect to SBP and FG levels in patients with manifested diabetes.

With respect to other potential modulating variables, sensitivity analyses and meta-regressions failed to reveal any correlations between the findings of the meta-analysis and age, sex, BMI and study duration (data not shown). These findings must be interpreted in a very conservative manner due to the low number of studies available for the meta-regressions. Not all the studies provided information on the quality of their respective set-up (e.g. method of randomisation and follow-up protocol with reasons for withdrawal; see online supplementary Fig. S1 for the risk of bias assessment according to the Cochrane Collaboration), demanding a conservative interpretation of results. In this context, it should be noted that the RCT varied with respect to dietary assessment methods to validate participant individual intakes. In addition, the drug regimen was not identical for all the participants in the included studies, and the diagnosis and classification of type 2 diabetes differed between the intervention trials. The pooled analysis of adiponectin values of only one study was disputable, but no more data were available. Part of the present meta-analysis was carried out using both post-intervention values and changes in MD; however, this was considered to be an acceptable procedure as described by the Cochrane Collaboration⁽¹²⁾.

This systematic review has some strengths as well. The meta-analysis was conducted following a stringent protocol; for example, the participants were randomly assigned to the intervention groups in all trials. RCT are considered to be the gold standard for evaluating the effects of an intervention and are subject to fewer biases when compared with observational studies.

In conclusion, HF diet consumption was found to exert beneficial effects on TAG levels, DBP and SBP, and HDL-C levels as well as FG levels in subjects who either were pre-diabetic or had manifested type 2 diabetes when compared with LF diet consumption. Therefore, HF and LF diets might not be of equal value in the management of either pre-diabetes or type 2 diabetes, leading to emphasis being placed on the recommendations of HF diets. In this regard, one major issue is the qualitative composition of fat (i.e. higher amounts of MUFA and PUFA and lower amounts of SFA in the percentage of TEC). As a large number of individuals with pre-diabetes or type 2 diabetes are either overweight or obese, nutritional recommendations often include hypoenergetic diets for weight management. Thus, a successful HF strategy has to implement limitations on other nutrients with energetic value, most probably carbohydrates. However, with respect to the high heterogeneity of the RCT included in this systematic review, further long-term intervention trials with a standardised approach

are necessary to elucidate the benefits and disadvantages of both dietary regimens.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0007114514000464>

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The authors' contributions are as follows: L. S. and G. H. conducted the data analysis, interpreted the results, drafted the manuscript and approved the final version of the manuscript. Both the authors read and approved the final manuscript. The authors have no conflicts of interest to declare.

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BMJ Open Dietary fatty acids in the secondary prevention of coronary heart disease: a systematic review, meta-analysis and meta-regression

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ABSTRACT

Objective: Previous systematic reviews were not restricted to either primary or secondary prevention trials, this study aimed to investigate the effects of reduced and/or modified fat diets and dietary fatty acids on all-cause mortality, cardiovascular mortality and cardiovascular events in participants with established coronary heart disease.

Design: Systematic review, meta-analysis and univariate/multivariate meta-regression.

Eligibility and criteria for selecting studies:

Electronic searches for randomised controlled trials comparing reduced/modified fat diets versus control diets were performed in MEDLINE, EMBASE and the Cochrane Library.

Data extraction: Pooled effects were calculated using an inverse-variance random effect meta-analysis. Random effects univariate and multivariate meta-regressions were performed including changes in all types of dietary fatty acids.

Results: Overall, 12 studies enrolling 7150 participants were included in the present systematic review. No significant risk reduction could be observed considering all-cause mortality (relative risk (RR) 0.92, $p=0.60$; $I^2=59\%$) and cardiovascular mortality (RR 0.96, $p=0.84$; $I^2=69\%$), combined cardiovascular events (RR 0.85, $p=0.30$; $I^2=75\%$) and myocardial infarction (RR 0.76, $p=0.13$; $I^2=55\%$) comparing modified fat diets versus control diets. This results could be confirmed for the reduced fat versus control diets (RR 0.79, $p=0.47$; $I^2=0\%$), (RR 0.93, $p=0.66$; $I^2=0\%$), (RR 0.93, $p=0.71$; $I^2=57\%$) and (RR 1.18, $p=0.26$; $I^2=18\%$). The multivariate and univariate model showed no significant associations between the independent variables and the changes from saturated fat, monounsaturated fat, polyunsaturated fat and linoleic acid. Sensitivity analyses did not reveal a significant risk reduction for any outcome parameter when polyunsaturated fat was increased in exchange for saturated fat.

Conclusions: The present systematic review provides no evidence (moderate quality evidence) for the beneficial effects of reduced/modified fat diets in the secondary prevention of coronary heart disease. Recommending higher intakes of polyunsaturated fatty acids in replacement of saturated fatty acids was not associated with risk reduction.

Strengths and limitations of this study

- Twelve studies enrolling 7150 participants were included in the present meta-analysis and meta-regression.
- Replacing saturated fatty acids by polyunsaturated fatty acids showed no significant benefit in the secondary prevention of coronary heart disease.
- Some of the included studies date back to 50 years.
- Substantial heterogeneity was observed for several outcomes.

INTRODUCTION

Studies reporting an association between intake of dietary saturated fatty acids (SFA) and serum cholesterol levels go back to the 1950s and 1960s.^{1 2} Supported by the epidemiological data observing a correlation between SFA intake and coronary heart disease (CHD) mortality, these findings established a reduction of SFA consumption as a major focus of dietary recommendations in order to prevent the prevalence of CHD³ although Siri-Tarino *et al*⁴ observed no relationship between saturated fats and CHD, stroke and cardiovascular disease (CVD) following a meta-analysis including 21 prospective studies.

Exchanging SFA for polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), carbohydrates (CHO) or protein exerted different effects on blood lipids and lipoproteins.⁵ In a systematic review and meta-analysis of cohort studies and randomised controlled trials (RCTs), Skeaff and Miller⁶ concluded that there is convincing evidence that replacement of SFA by PUFA decreases the risk of fatal CHD and CHD events; however they could not confirm the hypothesis of a direct association between SFA intake and CHD death.⁶ Furthermore,

the authors inferred that replacing SFA with CHO had no relation to CHD. The follow-up final report from the FAO stated that SFA intake should not be higher than 10% of the total energy consumption and that SFA should be replaced with PUFA.⁷ In their meta-analysis of cohort studies, Jakobsen *et al*⁸ observed that replacing SFA with PUFA reduced the risk of coronary events by 13% and the risk of coronary deaths by 26%, respectively. In contrast, replacement of SFA by CHO or MUFA marginally increased the risk of coronary events, whereas no significant effects on coronary death could be observed. Mozaffarian *et al*⁹ investigated the effects of increasing PUFA in replacement of SFA, and observed a significant decrease in the risk of CHD or associated mortality rates, while Hooper *et al*¹⁰ reported a reduction in cardiovascular risk subsequent to a long-term reduction or modification in dietary fat intake. In an update of their meta-analysis, the same research team suggested that lowering of SFA intake led to a 14% decrease of the risk of cardiovascular events, however without affecting the cardiovascular or total mortality rates.¹¹ It should be noted that reduction in cardiovascular risk was not associated with total fat in this study, but rather with a modification in dietary fat without clarifying the ideal type of unsaturated fat to replace SFA. Nevertheless, the US Dietary Guidelines recommend that <10% of total energy content (TEC) should come from SFA and that saturated fat should be replaced with MUFA and PUFA.¹² In 2011, the American Heart Association (AHA) and the American College of Cardiology (ACC) published a joint guideline endorsing less than 7% of TEC in the form of SFA for patients with coronary as well as other atherosclerotic vascular diseases.¹³ Since previous systematic reviews were not restricted to either primary or secondary prevention trials, this study aimed to investigate the effects of reduced/modified fat diets versus control diets on all-cause mortality, cardiovascular mortality, cardiovascular events (myocardial infarction, stroke) in participants with established CHD. The aim of the meta-regression was to include clinical outcomes and all dietary fatty acid changes in a univariate and multivariate model.

MATERIALS AND METHODS

Literature search

Queries of literature were performed using the electronic databases MEDLINE, EMBASE and the Cochrane Trial Register (until February 2014, respectively) with restrictions to RCTs, but no restrictions to language and calendar date using the following search terms: (*dietary fat OR fatty acids OR low fat diet OR modified fat diet*) in combination with (*secondary prevention OR cardiovascular disease OR myocardial infarction OR coronary heart disease*). Moreover, the reference lists from retrieved articles, systematic reviews and meta-analyses were checked to search for further relevant studies. This systematic review was planned, conducted and reported in adherence to

the standards of quality for reporting meta-analyses.¹⁴ Literature search was conducted independently by both the authors, with disagreements resolved by consensus.

Eligibility criteria

Studies were included in the meta-analysis if they met all of the following criteria: (1) randomised controlled design; (2) minimum intervention period with a follow-up of 12 months; (3) comparing a reduced fat (<30% of TEC) and/or modified fat diet versus control diet (SFA, MUFA, PUFA, linolenic acid and α -linolenic acid values were either extracted from intervention/dietary protocols or calculated from published data); (4) assessment of the 'outcome of interest' markers: all-cause mortality, cardiovascular mortality, combined cardiovascular events, myocardial infarctions (fatal and non-fatal); (5) report of the number of events and sample size for each group and (6) only participants with established CHD (survivor of myocardial infarction, stable/unstable angina pectoris, acute coronary insufficiency) or coronary artery disease (CAD, verified by coronary angiography).

Types of intervention

The focus of this systematic review was set on examining the effects of reduced/modified fat diets as compared with control diets on 'hard' clinical endpoints.

Risk of bias assessment and quality assessment

Full copies of studies were independently assessed for methodological quality by both the authors using the risk of bias assessment tool by the Cochrane Collaboration. The following sources of bias were detected: selection bias (random sequence generation, allocation concealment), performance/detection bias (blinding of participants and personnel, blinding of outcome assessment), attrition bias (incomplete data outcome), reporting bias (selective reporting) and other bias (figure 1).^{15 16}

Data extraction and statistical analysis

The following data were extracted from each study: the first author's last name, publication year, study duration, participant's sex and age, body mass index, sample size, SFA, MUFA, PUFA, linoleic acid, α -linolenic acid, dietary cholesterol content of intervention protocol or dietary protocol, caloric intake, information on supplements, primary outcomes, number of events. For each outcome measure of interest, a random-effects inverse-variance meta-analysis was performed in order to determine the pooled effect of the intervention in terms of relative risks (RRs) and number of events of the reduced fat versus control diets, and modified fat versus control groups. All data were analysed using the REVIEW MANAGER V.5.1 software, provided by the Cochrane Collaboration (<http://ims.cochrane.org/revman>). Heterogeneity between trial results was tested with a standard χ^2 test. The I^2 parameter was used to quantify

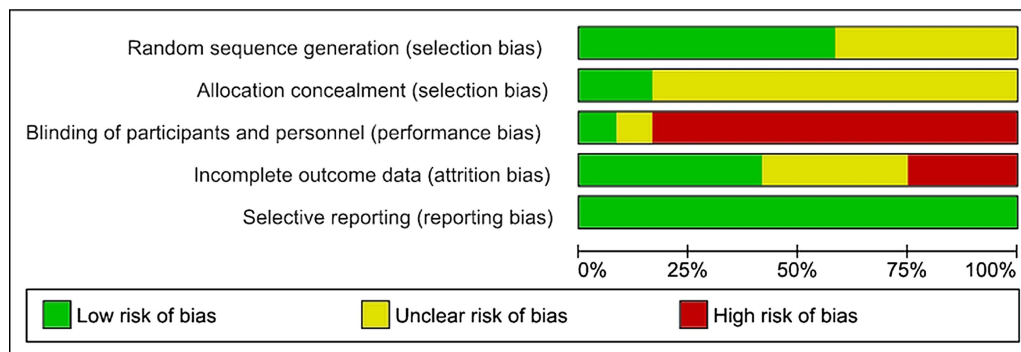


Figure 1 Risk of bias assessment tool. Across trials, information is either from trials at a low risk of bias (green), or from trials at unclear risk of bias (yellow), or from trials at high risk of bias (red). For each study, every bias domain will be checked, the given summary represents an assessment of bias risk across studies. For each bias domain, low risk of bias means that information is from studies at low risk of bias, high risk of bias indicates the proportion of information from studies at high risk of bias which might be sufficient to affect the interpretation of the results, and unclear risk of bias refers to information from studies at low or unclear risk of bias.

any inconsistency: $I^2 = [(Q - df) / Q] \times 100\%$, where Q is the χ^2 statistic and df is its degrees of freedom. A value for $I^2 > 50\%$ was considered to represent substantial heterogeneity.¹⁷ To consider heterogeneity, the random-effects model was used to estimate RRs and MDs with 95% CIs. Forest plots were generated to illustrate the study-specific effect sizes along with a 95% CI.

A random-effects univariate meta-regression was performed to examine the association between the change in percentage energy from SFA, PUFA (mixed n-6 and n-3), MUFA, as well as linoleic acid in the interventions versus control groups, and the dependent variables (log change RRs for all-cause mortality, CVD mortality, cardiovascular events and myocardial infarction). Furthermore, multivariate analyses were performed including all dietary fatty acid changes in a meta-regression model. As reported previously by Mensink *et al*,⁵ effects of protein (available only for five studies) and alcohol could not be estimated. The p values for differences in effects between the covariates were obtained using the *metareg* function of STATA V.12.0 (Stata-Corp, College Station, Texas, USA). Two sided p values < 0.05 were considered to be statistically significant. To determine the presence of publication bias, the symmetry of the funnel plots was assessed in which mean differences were plotted against their corresponding SEs. Sensitivity analyses were carried out to evaluate the influence of single trials on each meta-regression result. In addition, sensitivity analyses for PUFA versus SFA trials focusing on secondary prevention and sensitivity analysis for trials adopting 'fish advice' versus 'no fish advice' and combining reduced and modified fat diets were performed.

The quality of evidence was assessed according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines.^{18 19}

Missing data

Exposure data (SFA, PUFA, MUFA) for three study control groups^{20–22} were imputed based on average

background dietary intakes in similar populations at that time period, the corresponding data were derived from the National Diet Heart Study control group.²³ For evaluating linoleic acid, all of the trials that reported on total PUFA were included. Except for the Lyon Diet Heart Study, none of these studies had a major focus on n–3 fatty acids. Therefore, total PUFA in each of these other trials would be nearly all (90%+) linoleic acid. For studies giving information on the type of vegetable oil used, the proportion of linoleic acid and α -linolenic acid in total PUFA could be directly calculated.

RESULTS

Study characteristics

Altogether, 12 studies extracted from 2059 articles met the inclusion criteria and were included in the quantitative analysis.^{20–22 24–33} The study by Singh *et al*³¹ was included only in the sensitivity analysis, since this publication has been questioned for veracity.^{34 35} The detailed steps of the meta-analysis/meta-regression article selection process are described as a flow diagram in the online supplementary figure S1.

All studies included were RCTs with a duration ranging between 12 months and 6 years, published between 1965 and 2013 and enrolling a total of 6744 participants (7150 including the Singh trial). General and specific study characteristics are summarised in table 1 and online supplementary table S1, respectively.

All studies included participants with established CHD. Some trials had to be excluded due to various reasons: one study enrolled participants at high risk of CHD with only 50% of its participants suffering from CAD³⁶; another trial was not randomised³⁷; a recently performed secondary prevention RCT comparing a Mediterranean versus a low fat diet did not fulfil the inclusion criteria, since the intervention groups were not distinguished with respect to SFA intake³⁸; all secondary prevention studies with multifactorial

intervention protocols (eg, smoking cessation, better drug control, stress management or exercise) were excluded as well.^{39–44}

In the reduced/modified diet groups, the range for SFA varied between 7.2% and 14%, while the respective values in the control group varied between 11.7% and 26.4%. PUFA intakes were in the range 5–20.9%, MUFA ranged between 8% and 26%, and linoleic acid intake was at in the range 3.6–19.7% in the reduced/modified fat diet groups, respectively. Results of the univariate and multivariate meta-regression are summarised in [table 2](#).

Reduced versus control diets; modified fat versus control diets

With respect to clinical endpoints, no significant risk reduction could be observed considering all-cause mortality (RR 0.79 (95% CI 0.42 to 1.48), $p=0.47$; $I^2=0\%$) and cardiovascular mortality (RR 0.93 (95% CI 0.66 to 1.31), $p=0.66$; $I^2=0\%$), combined cardiovascular events (RR 0.93 (95% CI 0.65 to 1.34), $p=0.71$; $I^2=57\%$) and myocardial infarction (RR 1.18 (95% CI 0.88 to 1.59), $p=0.26$; $I^2=19\%$) comparing reduced fat versus control diets (see online supplementary figures S2–S5). Furthermore comparing modified fat versus control diets showed no significant effects on all-cause mortality (RR 0.92 (95% CI 0.68 to 1.25), $p=0.60$; $I^2=59\%$) and cardiovascular mortality (RR 0.96 (95% CI 0.65 to 1.42), $p=0.84$; $I^2=69\%$), combined cardiovascular events (RR 0.85 (95% CI 0.63 to 1.15), $p=0.30$; $I^2=75\%$) and myocardial infarction (RR 0.76 (95% CI 0.54 to 1.09), $p=0.13$; $I^2=55\%$) could be observed (see online supplementary figures S6–S9). Pooling reduced and modified fat diets all together resulted in no significant changes (see online supplementary figures S10–S13).

Univariate meta-regression

Taken together, the univariate meta-regression showed no significant association between changes in SFA, PUFA, MUFA, linoleic acid and risk of all-cause mortality, cardiovascular mortality, total cardiovascular events and myocardial infarction (see online supplementary figures S14–S29).

Multivariate meta-regression

Similar to the univariate model, the multivariate meta-regression did not reveal any significant association between changes in SFA, PUFA and MUFA and risk of all-cause mortality, cardiovascular mortality, cardiovascular events and myocardial infarction.

Sensitivity analyses

Sensitivity analyses were performed to evaluate the influence of single trials on each meta-regression. None of the trials had a significant impact on the results of the univariate and multivariate meta-regression.

An additional sensitivity analysis was carried out according to the main analysis of Mozaffarian *et al*⁶ as well as Skeaff and Miller,⁶ evaluating the replacement of

SFA by PUFA (including the new data of the Sydney Diet Heart Study³³). The results showed that replacing SFA by PUFA was not associated with a significant risk reduction for all-cause mortality, cardiovascular mortality, combined cardiovascular events and myocardial infarction (see online supplementary figures S30–S33) in participants with established CHD/CAD.

Another sensitivity analysis was performed including only those trials recommending a higher consumption of fatty fish. This resulted in a significant reduction of cardiovascular events, cardiovascular mortality and all-cause mortality (see online supplementary figures S34–S36).

Since in the Sydney Diet Heart Study a commercial margarine probably high in transfatty acids was used to deliver PUFA to the intervention group, a sensitivity analysis was performed excluding this trial. However, all results of the primary analysis could be confirmed.

Publication bias

The funnel plots indicate moderate asymmetry, suggesting that publication bias cannot be completely excluded as a factor of influence on the present meta-analysis (see online supplementary figures S37–S40).

Overall quality of evidence

The overall quality of evidence rated according to the GRADE guidelines for all outcomes was moderate.

DISCUSSION

In this systematic review of 7150 participants with established CHD or CAD comparing reduced and/or modified fat diets versus control diets, no significant risk reduction (moderate quality of evidence) for all-cause mortality, cardiovascular mortality, cardiovascular events and myocardial infarction could be observed. Since no previous meta-analysis compared the effects of reduced and/or modified fat diets as a means for secondary prevention, the present data will be discussed together with results of combined primary/secondary prevention trials.

Small but nevertheless significant reductions in cardiovascular events could be observed by Hooper *et al*⁴⁵ as well as Truswell⁴⁶ following their respective meta-analyses of intervention studies investigating the effects of a modification of fatty acid intake as a secondary preventive measure in patients with CVD. Systematic reviews analysing trials targeted both at primary and secondary prevention found that replacing SFA with unsaturated fatty acids reduced cardiovascular events.¹¹ The question whether these benefits are due to CHO, MUFA or due to PUFA is discussed controversially. The systematic review by Skeaff and Miller observed a significant increase in risk (by 25%) of CHD death in the highest category of dietary PUFA. In contrast, pooling RCTs indicated that a 5% increase in PUFA intake was associated with a significant reduction in CHD events.⁶ Mente *et al*⁴⁷ observed a

Table 1 General study characteristics

Reference	Sample size, baseline BMI (kg/m ²)	Age (years), female (%) male (%)	Duration (years)	SFA/PUFA/MUFA/trans FA (as indicated by the investigators)	LA, ALA	Fish consumption advice	TF, cholesterol	Inclusion criteria	Supplement	Energy amount (end of the study)	Outcomes
Ball <i>et al</i> (1965) ^{20*}	264 nd	<65 100%	4	I: 9%, 5%, 8% C: 16–18%, 7%, 15%	I: 4.5% C: 6.3%		I: 22%, 330 mg C: 47%, 650–750 mg	Post-MI	–	2030 2360	ACM, CVM, CVE, MI
Burr <i>et al</i> (1989) ²⁷	2033	56.6 100%	2	I: 11.3%, 8.9%, 12.1% C: 15%, 6.4%, 13.6%	I: 8.01% C: 5.76%		I: 32.3% C: 35%, 650–750 mg	Post-MI	–	nd	ACM, CVM, CVE
de Lorgeril <i>et al</i> (1994) ²⁴	605 nd	53.5 9.25% 90.75%	4	I: 8.3%, 8.2%, 12.9% C: 11.7%, 11.4%, 10.3%	I: 3.6%, 0.81% C: 5.3%, 0.27%	yes	I: 30.5%, 217 C: 32.7%, 318 mg	Post-MI	I: Canola oil based margarine	1928 2140	ACM, CVM, CVE
Howard <i>et al</i> (2006) ³²	2277 29.1	62.3 100%	6	I: 9.5%, 6.1%, 10.8%, 1.8% C: 12.4%, 7.5%, 14.2%, 2.4%	I: 5.49% C: 6.75%		I: 28.8%, 193.6 mg C: 37%, 243.5 mg	Post-MI, stroke, CABG or PCI	–	1431 1546	CVM, CVE, MI, stroke
Leren <i>et al</i> (1970) ^{21*}	412 nd	56.25 100%	5	I: 8.5%, 20.7%, 10.1% C: 16–18%, 7%, 15%	I: 14.8%, 2.7% C: 3.3%, nd	yes	I: 39%; 264 mg C: 40%, 650–750 mg	Post-MI	I: 75 g Soya-bean oil	2380	ACM, CVM, CVE, MI
MRC (1968) ²⁶	393 nd	53.5 100%	5	I: 11.3%, 20.4%, 14.3% C: 26.4%, 4.4%, 12.2%	I: 16.3%, 2.3% C: 3.96		I: 46% C: 43%, 650–750 mg	Post-MI	I: 85 g soya-bean oil	nd nd	ACM, CVM, CVE, MI
Michalsen <i>et al</i> (2006) ²⁹	101 26.55	59.4 23% 77%	1	I: 10.1%, 6.1%, 10.6% C: 13%, 7.4%, 12.2%	I: 4.8%, 0.72% C: 6.2%, 0.7%	yes	I: 32.2%, 251 C: 35.2%, 265 mg	CAD verified by coronary angiography	–	2241 2237	–
Rose <i>et al</i> (1965) ²²	80 nd	<70 100%	2	I: instructed to avoid fried foods, fatty meats, sausages, pastry,	I-olive: 8.2%, 0.28% I-corn: 19.7%, 0.07% C: 6.3%		I-olive: 46.2% I-corn: 50.5% C: 32.5%	Post-MI, angina pectoris	I: 80 g olive oil; 80 g corn oil	2045 2070 1933	ACM, CVM, CVE, MI

Continued

Table 1 Continued

Reference	Sample size, baseline BMI (kg/m ²)	Age (years), female (%) male (%)	Duration (years)	SFA/PUFA/MUFA/trans FA (as indicated by the investigators)	LA, ALA	Fish consumption advice	TF, cholesterol	Inclusion criteria	Supplement	Energy amount (end of the study)	Outcomes
				ice-cream, cheese, cakes, butter, milk, eggs restricted I-olive: 11.7%, 8.7%, 26% I-corn: 14%, 21.85%, 14.9% C: no advice on dietary fat C: 16–18%, 7%, 7%							
Singh <i>et al</i> (1992) ³¹	406 23.8	51.25 12% 88%	1	I: 7.2%, 8.6%, 8% C: 10.8%, 7%, 10.2%	I: 7.74% C: 6.3%	yes	I: 23.8% C: 28%	Post-MI, unstable angina pectoris	–	1810 1940	ACM, CVM, CVE, MI
Sondergaard <i>et al</i> (2003) ³⁰	131 nd	62.5 30% 70%	1	I: 10.3%, 7.5%, 8.4% C: 12%, 6.8%, 10.1%	I: 6.75% C: 6.3%	yes	I: 26.2% C: 28.9%	Post-MI, stable/unstable angina pectoris	–	1520 1550	–
Watts <i>et al</i> (1992) ²⁸	90 26.25	51.4 100%	3	I: 8–10%, 8%, 10%, 1.08% C: 17%, 5.2%, 14.8%, 1.8%	I: 5%, 0.32% C: 3.9%, 0.41%		I: 27% C: 37%	Angina pectoris	–	nd	ACM, CVM, CVE, MI
Woodhill <i>et al</i> (1978) ²⁵ Ramsden <i>et al</i> (2013) ³³	458 nd	49 100%	5	I: 9.8%, 15.2%, 11.5% C: 13.5%, 8.9%, 13.8%	I: 15%, nd C: 8%, nd		I: 38.3%, 248 C: 38.1%, 342	Post-MI, angina pectoris, coronary insufficiency	–	2289 2191	ACM, CVM, CVE

*SFA, MUFA and PUFA for study control groups were imputed based on average background dietary intakes in similar populations of the Diet Heart Study control group of 1968.²⁶

ACM, all-cause mortality; ALA, α -linolenic acid; BMI, body mass index; C, control; CABG, coronary artery bypass graft; CAD, coronary artery disease; CVE, cardiovascular events; CVM, cardiovascular mortality; FA, fatty acids; HDL-C, high-density lipoprotein cholesterol; I, intervention; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; MUFA, monounsaturated fat; nd, no data; PCI, percutaneous coronary intervention; PUFA, polyunsaturated fat; SFA, saturated fat; TC, total cholesterol; TF, total fat; TG, triacylglycerols.

Table 2 Univariate and multivariate meta-regression for change in dietary fatty acid (covariate=percentage energy change in SFA, PUFA, MUFA and LA between intervention vs control groups)

Covariate	Number of studies or subsets	β -Coefficient	95% CI	p Value	Heterogeneity (I ²) (%)
All-cause mortality (univariate)					
SFA	8	0.0029	−0.1156 to 0.1216	0.953	75.24
PUFA	8	0.0253	−0.0351 to 0.0858	0.345	72.97
MUFA	8	−0.0024	−0.1475 to 0.1426	0.968	72.04
LA	8	0.0355	−0.0387 to 0.1099	0.286	73.04
Cardiovascular mortality (univariate)					
SFA	9	0.0089	−0.0900 to 0.1079	0.836	56.89
PUFA	9	0.0182	−0.0449 to 0.0813	0.517	58.23
MUFA	9	0.0186	−0.1428 to 0.1801	0.793	56.58
LA	9	0.0245	−0.0517 to 0.1008	0.472	58.10
Total cardiovascular events (univariate)					
SFA	9	0.0181	−0.0884 to 0.1247	0.699	70.88
PUFA	9	0.0157	−0.0444 to 0.0759	0.556	73.70
MUFA	9	−0.0069	−0.1172 to 0.1033	0.885	75.80
LA	9	0.0224	−0.0492 to 0.0940	0.484	74.05
Myocardial infarction (univariate)					
SFA	9	0.0114	−0.0813 to 0.1041	0.780	66.23
PUFA	9	0.0011	−0.0529 to 0.0551	0.962	66.60
MUFA	9	−0.0224	−0.1267 to 0.0818	0.627	69.19
LA	9	0.0028	−0.0627 to 0.06840	0.922	66.87
All-cause mortality (multivariate)					
SFA	8	0.0800	−0.1390 to 0.2991	0.368	77.39
PUFA	8	0.0568	−0.0534 to 0.1671	0.226	
MUFA	8	−0.0230	−0.2254 to 0.1793	0.768	
Cardiovascular mortality (multivariate)					
SFA	9	0.0768	−0.1422 to 0.2959	0.409	67.14
PUFA	9	0.0584	−0.0660 to 0.1830	0.281	
MUFA	9	−0.0381	−0.2691 to 0.1927	0.689	
Total cardiovascular events (multivariate)					
SFA	9	0.1103	−0.0691 to 0.2898	0.175	77.96
PUFA	9	0.0638	−0.0347 to 0.1623	0.157	
MUFA	9	−0.0825	−0.2417 to 0.0765	0.240	
Myocardial infarction (multivariate)					
SFA	9	0.0665	−0.1237 to 0.2568	0.410	75.28
PUFA	9	0.0355	−0.0703 to 0.1414	0.428	
MUFA	9	−0.0675	−0.2424 to 0.1072	0.366	

LA, linoleic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

significant inverse correlation between MUFA (but not PUFA) intake and CHD events, thus favouring MUFA as a mediator for the beneficial effects of reduced SFA intake. Furthermore, a review of 16 meta-analyses indicated that a diet rich in MUFA has several beneficial effects on a broad range of CVD risk factors, in the primary prevention of CVD.⁴⁸

In contrast to the data presented in this systematic review, a recent meta-analysis of observational studies suggests that replacing SFAs with PUFAs may have a greater benefit than replacing SFAs with CHO or MUFA.⁸ Moreover, the meta-analysis of RCTs by Mozaffarian *et al*⁹ reported a significant reduction in CHD events (by 19%) following the replacement of SFA by PUFA. However, adapting (for secondary prevention) and updating (including new data from the Sydney Diet Heart Study) the meta-analysis by Mozaffarian *et al*,⁹ as

was carried out in a sensitivity analysis investigating the replacement of SFA by PUFA in the present study, resulted in neither beneficial nor detrimental effects on all outcome parameters. Compared with meta-analyses of observational studies, those of RCTs are considered to have a higher grade of quality.¹⁸ RCTs of lifestyle behaviours such as diet are often limited by lack of double blinding, non-compliance, cross-over and dropout—as evidenced by the trials in the current meta-analysis—so that well-designed analyses in prospective cohort studies provide important evidence with complementary strengths and limitations.

No detrimental effects of increased amounts of linoleic acid could be observed in meta-regression. The results of the present meta-regression are in discrepancy with the observations of a recent meta-analysis of Ramsden *et al*,³³ providing evidence that replacement of

SFA with linoleic acid was associated with increased rates of death from all causes, CHD and CVD, respectively. In order to rationalise their results, Ramsden *et al* proposed a mechanistic model linking dietary linoleic acid to CVD pathogenesis. It is proposed that diets high in n-6 linoleic acid facilitate production of oxidised linoleic acid metabolites mediating progression of atherosclerosis and thus leading to higher rates of cardiovascular mortality.³³ Owing to the low number of studies available and the inherent biases of the method, the findings of the present meta-regressions must be interpreted in a very conservative manner. However, when using these results to generate a hypothesis, it still seems reasonable to replace SFA by PUFA in the secondary prevention of CHD, although SFA should not be completely substituted by n-6 fatty acids as recommended by the FAO.⁷ Instead, dietary advice should rather focus on increasing the uptake of n-3 fatty acids, predominantly in the form of fatty fish.

The present systematic review does not consider unpublished data, and it cannot be excluded that these results may have had at least a moderate impact on the effect size estimates. Examination of funnel plots showed little to moderate asymmetry suggesting that publication bias cannot be completely excluded as a confounder of the present meta-analysis (eg, it remains possible that small studies yielding inconclusive data have not been published). Another limitation of nutritional intervention trials is the heterogeneity of various aspects and characteristics of the study protocols. RCTs in the present analysis varied with respect to the type(s) of diets used (eg, advised to supplement various oils, fatty fish), and size of study population. Some of the included studies date back to 50 years, and not all of the studies provided information on the quality of their respective setup (eg, method of randomisation, follow-up protocol with reasons for withdrawal, see figure 1 for risk of bias assessment according to the Cochrane Collaboration), again demanding a conservative interpretation of results. Another potential limitation of the present meta-analysis is rather specifically related to the research question. Total fat intake in the reduced and/or modified diet groups ranged between 20% and 50% of TEC. In some trials, dietary fat intake was established by adding corn, olive or soybean oil,^{21 26} while others implemented canola oil-based margarine.²⁴ In several trials, the participants in the reduced and/or modified fat groups were provided with additional dietary advice such as to increase the consumption of fatty fish,^{21 24 29–31} vegetables and fruits,^{24 29–31} or nuts³¹ as well as to pay attention to their cholesterol intake. In contrast to these heterogeneous aspects of the study designs, length of trials included in this systematic review was rather homogenous with all RCTs having a running time of at least 1 year. However, this might be interconnected with another major limitation of dietary intervention trials, that is, the issue of compliance. Participants in the different intervention groups may not exactly adhere to the advised or

prescribed dietary protocol. In the end, this may not only lead to deviations from the target values but may result in only minimal differences between the study arms.

CONCLUSION

The present meta-analyses and meta-regressions provide no evidence for a beneficial secondary preventive effect of either reduced and/or modified fat diets or replacement of SFA by PUFA in participants with established CVD. Although pharmaceutical treatment via lipid-lowering medications is considered to be an effective therapeutic measure for patients with established CVD, many international authorities recommend modifications of fat intake with a special emphasis on SFA as well.^{13 49 50} The current AHA/ACC guidelines promote to reduce the total fat intake to less than 35% of TEC and SFA intake to less than 7% of TEC, respectively. However, recommending higher intakes of PUFA in general instead of SFA might not be appropriate. Sensitivity analysis indicates that it seems reasonable to modify this general recommendation by promoting higher dietary n-3 PUFA as a substitute for SFA.

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Dietary fatty acids in the secondary prevention of coronary heart disease: a systematic review, meta-analysis and meta-regression

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RESEARCH

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Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies

Lukas Schwingshackl* and Georg Hoffmann

Abstract

Background: The aim of the present meta-analysis of cohort studies was to focus on monounsaturated fat (MUFA) and cardiovascular disease, cardiovascular mortality as well as all-cause mortality, and to distinguish between the different dietary sources of MUFA.

Methods: Literature search was performed using the electronic databases PUBMED, and EMBASE until June 2nd, 2014. Study specific risk ratios and hazard ratios were pooled using a inverse variance random effect model.

Results: Thirty-two cohort studies (42 reports) including 841,211 subjects met the objectives and were included. The comparison of the top versus bottom third of the distribution of a combination of MUFA (of both plant and animal origin), olive oil, oleic acid, and MUFA:SFA ratio in each study resulted in a significant risk reduction for: all-cause mortality (RR: 0.89, 95% CI 0.83, 0.96, $p = 0.001$; $I^2 = 64\%$), cardiovascular mortality (RR: 0.88, 95% CI 0.80, 0.96, $p = 0.004$; $I^2 = 50\%$), cardiovascular events (RR: 0.91, 95% CI 0.86, 0.96, $p = 0.001$; $I^2 = 58\%$), and stroke (RR: 0.83, 95% CI 0.71, 0.97, $p = 0.02$; $I^2 = 70\%$). Following subgroup analyses, significant associations could only be found between higher intakes of olive oil and reduced risk of all-cause mortality, cardiovascular events, and stroke, respectively. The MUFA subgroup analyses did not reveal any significant risk reduction.

Conclusion: The results indicate an overall risk reduction of all-cause mortality (11%), cardiovascular mortality (12%), cardiovascular events (9%), and stroke (17%) when comparing the top versus bottom third of MUFA, olive oil, oleic acid, and MUFA:SFA ratio. MUFA of mixed animal and vegetable sources per se did not yield any significant effects on these outcome parameters. However, only olive oil seems to be associated with reduced risk. Further research is necessary to evaluate specific sources of MUFA (i.e. plant vs. animal) and cardiovascular risk.

Keywords: Monounsaturated fatty acids, Olive oil, Cohort studies, Meta-analysis, Cardiovascular disease

Background

The most common monounsaturated fatty acids (MUFA) in daily nutrition is oleic acid, followed by palmitoleic acid, and vaccenic acid. Moreover, oleic acid represents the top-most MUFA provided in the diet (~90% of all MUFA). No dietary recommendations for MUFA are given by the National Institute of Medicine, the United States Department of Agriculture, the European Food and Safety Authority and the American Diabetes Association. In contrast, the Academy of Nutrition and Dietetics as well as the Canadian Dietetic Association both promote <20%

MUFA of daily total energy consumption, while the American Heart Association sets a limit of 20% MUFA in their respective guidelines [1-3]. One reason for specific MUFA recommendations might be their potential benefit in the primary and secondary prevention of cardiovascular diseases. However, previous meta-analyses of cohort studies reported inconsistent results of MUFA on coronary heart disease (CHD). Jakobsen et al. [4] observed that replacement of SFA by MUFA marginally increased the risk of coronary events, whereas no significant effects on coronary death could be observed. These results are in strong discrepancy with another meta-analysis of cohort studies, where Mente et al. [5] reported a significant correlation between MUFA intake and a decrease in the relative risk for

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CHD. Skeaff and Miller [6] did not observe any effects of MUFA-rich diets on relative risks of CHD events and CHD death. Likewise, the most recent meta-analysis by Chowdhury et al. including nine cohort studies found no significant associations between MUFA intake, circulating MUFA and risk of CHD [7].

One explanation for these inconclusive data might be that different sources of MUFA were not taken into account. Adopting a western diet means that MUFA is predominantly supplied by foods of animal origin, while in south European countries, extra virgin olive oil is the most dominant source of this type of fatty acid [8]. Results of the recently published PREDIMED trial demonstrated major cardiovascular benefits of olive oil and nuts when compared to a low-fat diet [9]. As a major outcome parameter, the risk of stroke was reduced, an event which has not been included in the meta-analyses mentioned above. In addition, a recent cohort study observed a significant association between dietary olive oil, higher plasma oleic acid and reduced risk of stroke [10]. Extra virgin olive oil is regarded to be the genuine driver of the Mediterranean diet and was found to be associated with a 26% reduced risk of all-cause mortality in the Spanish branch of the EPIC study [11]. The aim of the present meta-analysis of cohort studies was to focus on MUFA and CVD (combining CHD and stroke), cardiovascular mortality, and all-cause mortality, and to distinguish between the different dietary sources of MUFA (e.g. olive oil).

Materials and methods

Literature search

Queries of literature were performed using the electronic databases PUBMED, and EMBASE (until 2nd June 2014, respectively) with no restrictions to language, and calendar date using the following search terms: (*"dietary fat" OR "fatty acids" OR "monounsaturated fat" OR "mufa" OR "olive oil" OR "oleic acid" OR "mediterranean diet"*) AND (*"cardiovascular disease" OR "myocardial infarction" OR "coronary heart disease" OR "stroke" OR "mortality"*) AND (*"incidence" OR "cohort" OR "follow-up" OR "prospective" OR "risk ratio" OR "hazard ratio" OR "rate ratio"*). Moreover, the reference lists from retrieved articles, systematic reviews, and meta-analyses were checked to search for further relevant studies. This systematic review was planned, conducted, and reported in adherence to standards of quality for reporting meta-analyses [12]. Literature search was conducted independently by both authors, with disagreements resolved by consensus.

Eligibility criteria

Studies were included in the meta-analysis if they met all of the following criteria: (i) cohort study design; (ii) data related to dietary consumption of MUFA, MUFA:

SFA ratio, olive oil, and oleic acid; (iii) the primary outcomes were: all-cause mortality, CVD mortality, combined CVD events (cardiovascular mortality, cardiovascular morbidity (non-fatal myocardial infarction, angina, stroke, heart failure, peripheral vascular events)); the secondary outcomes were: coronary heart disease, and stroke; (iv) adjusted relative risks (RRs), and hazard ratios (HRs) with corresponding 95% confidence intervals (95% CIs) or the data necessary to calculate these; (v) when a study appeared to have been published in duplicate, the version containing the most comprehensive information was selected.

Data extraction and quality assessment

The following data were extracted from each study: the first author's last name, year of publication, study origin, outcome parameter, sample size, study length, age at entry, sex, specification of MUFA, adjustment factors, quality score, and risk estimates (HR, RR; highest vs. lowest category) with their corresponding 95% CIs. If separate risk estimates for males and females or separate risk estimates for ages were available in one study, the data were pooled and treated as one study. When a study provided several risk estimates, the maximally adjusted model was chosen. To assess the study quality, a 9-point scoring system according to the Newcastle-Ottawa Scale (NOS) was used. Hence, the full score was 9, and a high-quality study in the present analysis was defined by a threshold of ≥ 7 points [13]. Data extraction and quality assessment were performed by one author (L.S.).

Statistical analysis

The meta-analysis was performed by combining the multivariable adjusted RR or HR of the highest compared with the lowest MUFA, MUFA:SFA ratio, oleic acid, or olive oil category based on random effects model using DerSimonian-Laird method, which incorporated both within and between study variability [14]. To ensure a transparent approach to meta-analysis and interpretation of findings in this review, RR/HR estimates for association of fatty acids and primary/secondary outcomes that were often differently reported by each study (such as per-unit or per-1-SD change or comparing quintiles, quartiles, thirds, and other groupings) were transformed, using methods previously described [7]. These transformed estimates consistently corresponded to the comparison of the top versus bottom third of MUFA, MUFA:SFA ratio, olive oil, and oleic acid distribution in each study. To evaluate the weighting of each study, the standard error for the logarithm HR/RR of each study was calculated and regarded as the estimated variance of the logarithm HR/RR using an inverse variance method [14]. Studies were grouped according to the different clinical outcomes (all-cause mortality,

cardiovascular mortality, combined cardiovascular events, coronary heart disease, and stroke). Subgroup analysis was performed for total MUFA, MUFA:SFA ratio, oleic acid, and olive oil. Heterogeneity was estimated by the Cochrane Q test together with the I^2 statistic. An I^2 value >50% indicates substantial heterogeneity across studies [15]. The *heterogi* command in STATA was used to calculate the confidence intervals for the heterogeneity estimates. Funnel plots were used to assess potential publication bias. To determine the presence of publication bias, we assessed the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors. In addition, Egger test was performed to test for potential publication bias [16]. Sensitivity analyses were performed assuming statistical heterogeneity with the *metaan* command in STATA [17]. All analyses were conducted using the Review Manager by the Cochrane Collaboration (version 5.2) and STATA 13.0 (Stata-Corp, College Station, TX).

Missing data

Dr. Goldbourt (personal communication) provided the 23 year follow-up all-cause mortality and cardiovascular mortality data of the Israeli civil cohort for the highest vs. lowest quintile MUFA: SFA ratio [18].

Results

Literature search and study characteristics

A total of 32 cohort studies (42 reports) met the inclusion criteria and were included in the meta-analysis [10,11,18-57]. Full search strategy for PUBMED is given in the Additional file 1. General study characteristics are given in Table 1. Sample size varied between 161 and 161,808 with a follow-up time ranging from 3.7 to 30 years. The total number of subjects in the included studies was 841,211.

Main outcomes

According to the different clinical outcomes, overall risk of all-cause mortality was evaluated in seventeen cohorts, cardiovascular mortality in fourteen cohorts, combined cardiovascular events in twenty-eight cohort studies, coronary heart disease in fifteen cohorts, and stroke in eleven cohorts, respectively.

Random effects model data (as summarized in Table 2) revealed that top versus bottom third combined MUFA, olive oil, oleic acid, and MUFA:SFA ratio was significantly associated with a reduced risk of: all-cause mortality (relative risk, RR: 0.89, 95% confidence interval 0.83 to 0.96; $p = 0.001$, $I^2 = 64\%$) (Figure 1), cardiovascular mortality (RR: 0.88, 95% CI 0.80 to 0.96, $p = 0.004$, $I^2 = 50\%$) (Figure 2), combined cardiovascular events (RR: 0.91, 95% CI 0.86 to 0.96, $p = 0.001$, $I^2 = 58\%$) (Figure 3), and stroke (RR: 0.83, 95% CI 0.71 to 0.97, $p = 0.02$, $I^2 = 70\%$). In

contrast, no significant changes could be observed for coronary heart disease (RR: 0.96, 95% CI 0.90 to 1.01, $p = 0.13$, $I^2 = 41\%$).

Subgroup/sensitivity analyses

Following subgroup analyses, olive oil most likely turned out to be crucial for the results of the primary analysis, since significant associations could only be found between higher intakes of olive oil and reduced risk of all-cause mortality (RR: 0.77, 95% CI 0.71 to 0.84, $p < 0.00001$, $I^2 = 0\%$), cardiovascular events (RR: 0.72, 95% CI 0.57 to 0.91, $p = 0.007$, $I^2 = 77\%$), and stroke (RR: 0.60, 95% CI 0.47 to 0.77, $p < 0.0001$, $I^2 = 0\%$), respectively. Subgroup analysis for MUFA (of mixed animal and plant origin) did not reveal any significant risk reduction for all-cause mortality (RR: 1.00, 95% CI 0.93 to 1.08, $p = 0.93$, $I^2 = 23\%$), cardiovascular mortality (RR: 0.95, 95% CI 0.89 to 1.02, $p = 0.14$, $I^2 = 52\%$), cardiovascular events (RR: 0.96, 95% CI 0.89 to 1.04, $p = 0.36$, $I^2 = 7\%$), coronary heart disease (RR: 0.99, 95% CI 0.93 to 1.06, $p = 0.76$, $I^2 = 29\%$), and stroke (RR: 0.85, 95% CI 0.72 to 1.01, $p = 0.07$, $I^2 = 65\%$). To investigate statistical heterogeneity, sensitivity analyses were performed with the *metaan* command in STATA. Heterogeneity of the main analysis could be confirmed in the sensitivity analyses. Differentiating between studies performed in Europe vs. non-European investigations resulted in significant differences as compared to the main analysis. Pooling European based cohorts resulted in a significant risk reduction for all-cause mortality (RR: 0.87, 95% CI 0.79 to 0.95) as well as for cardiovascular mortality (RR: 0.76, 95% CI 0.64 to 0.91) and cardiovascular events (RR: 0.86, 95% CI 0.78, 0.95). In contrast, no significant reduction in all-cause mortality risk (RR: 0.97, 95% CI 0.91 to 1.04) could be observed for non-European cohorts (the respective data for cardiovascular mortality being RR: 0.94, 95% CI 0.89 to 0.99 and for cardiovascular events being RR: 0.93, 95% CI 0.87 to 0.98). With respect to study length, studies with a follow-up ≥ 10 years resulted in similar results as compared to short-term studies (<10 years follow up). Likewise, high quality studies could confirm the results of the primary analysis.

Publication bias

The Egger's linear regression tests provided evidence for a potential publication bias for combined cardiovascular events ($p = 0.018$), all-cause mortality ($p = 0.041$), and cardiovascular mortality ($p = 0.12$) following comparison of the top versus bottom third combined MUFA, olive oil, oleic acid, and MUFA:SFA ratio. No evidence of publication bias could be detected for risk of CHD ($p = 0.28$) and stroke ($p = 0.28$). All funnel plots indicate little to moderate asymmetry, suggesting that publication bias cannot be completely excluded as a factor of influence on the present meta-analysis (Additional file 1: Figures S1,

Table 1 General study characteristics of the included cohort studies

Author, year	Cohort name country	Outcomes	Population	Follow-up (years)	Age at entry (years)	Sex	MUFA source	Adjustment	Multivariate adjusted	Study quality (Newcastle-Ottawa scale)
Atkins et al. 2014 [34]	British Regional Heart Study GBR	All-cause mortality CVD mortality CVD events CHD events	3,328	11.3	60-79	M	Olive oil	Age, energy intake, smoking, alcohol, PA, social class, BMI, and a modified version of the HDI/EDI score not containing the individual component of interest	Fourth vs. first quartile	8
Barzi et al. 2003 [50]	Studio della Sopravvivenza nell'Infarto Miocardico ITA	All-cause mortality	11,323 with myocardial infarction	6.5	59	M/F	Olive oil	Age, sex, hypertension, HDL-cholesterol, diabetes, smoking, claudication, electrical instability, left ventricular dysfunction, residual myocardial ischaemia, dietary supplementation, pharmacological therapies	Third vs. first tertile	7
Bendinelli et al. 2011 [49]	European Prospective Into Cancer and Nutrition ITA	CHD	29,689	7.85	35-74	F	MUFA MUFA:SFA	Energy intake, educational level, smoking status, alcohol consumption, body height, body weight, waist circumference daily non-alcohol caloric intake, hypertension, menopausal status, PA, total meat consumption	Fourth vs. first quartile	8
Buckland et al. 2012 [11]	European Prospective Into Cancer and Nutrition SPA	All-cause mortality CVD mortality Cancer mortality	41,078	10.4	29-69	M/F	Olive oil	Centre, sex, age, energy intake, BMI, waist circumference, educational status, smoking status, PA, and alcohol intake, intake of fruit, vegetables, meat, and dairy	Fourth vs. first quartile	8
Buckland et al. 2012 [56]	European Prospective Into Cancer and Nutrition SPA	CHD	40,142	10.4	29-69	M/F	Olive oil	Educational level, BMI, waist circumference, PA, smoking status, alcohol consumption, energy intake excluding alcohol, hyperlipidaemia, hypertension and diabetes, Mediterranean diet score (excluding olive oil and alcohol)	Fourth vs. first quartile	8
Chiuve et al. 2012 [32]	Nurses' Health Study USA	Sudden death	91,981	30	34-59	F	MUFA	Total calories, smoking, BMI, family history of myocardial infarction, menopausal status, hormone therapy, exercise, aspirin use, use of multivitamins, use of vitamin E supplements, alcohol use, and history of diabetes, hypertension, hypercholesterolemia, coronary heart disease, and cancer at baseline, percentage of energy from total fat	Fifth vs. first quintile	8
Dilis et al. 2012 [19]	European Prospective Into Cancer and Nutrition GRE	CHD mortality CHD incidence	23,929	10	20-86	M/F	MUFA Olive Oil MUFA:SFA	Age, BMI, height, PA, years of schooling and energy intake entered, alcohol consumption, smoking status and arterial blood pressure	1 SD increment	9
Esrey et al. 1996 [42]	Lipid Research Clinics Prevalence Study USA	CVD mortality	4,546	12.4	≥30	M/F	MUFA	Age, sex, energy intake, serum lipids, systolic blood pressure, cigarette smoking, BMI, glucose intolerance	1 unit increase	9

Table 1 General study characteristics of the included cohort studies (Continued)

Gardener et al. 2011 [43]	Northern Manhattan Study USA	Ischemic stroke Myocardial infarction Vascular death	2,568	9	>40	M/F	MUFA:SFA	Age, sex, race ethnicity, completion of high school education, moderate-to-heavy PA, energy intake, and cigarette smoking	≥ median vs. < median	7
Gillman et al. 1997 [40]	Framingham Heart Study USA	Stroke	832	20	45-65	M	MUFA	Age, energy, systolic blood pressure, cigarette smoking, glucose intolerance, BMI, PA, left ventricular hypertrophy, alcohol, fruit and vegetables	1% increase MUFA	9
Goldbourt et al. 1993 [18]	Israeli Ischemic Heart Disease Study ISR	CHD All-cause mortality	10,059	23	>40	M	MUFA:SFA	Age	Fifth vs. first quintile	7
Guasch-Ferre et al. 2014 [54]	PREvención con Dieta MEDiterránea SPA	All-cause mortality CVD mortality CVD events	7216	4.8	55-80	M/W	Olive oil	Age, sex, BMI, smoking status, alcohol intake, education level, PA, prevalence of diabetes, prevalence of hypertension, prevalence of hypercholesterolemia, use of antihypertensive, use of statins, Mediterranean diet adherence	Third vs. first tertile	8
He et al. 2003 [47]	Health professional study USA	Stroke	43,732	14	40-75	M	MUFA	BMI, PA, history of hypertension, smoking status, aspirin use, multivitamin use, and consumption of alcohol, potassium, fibre, and vitamin E, total servings of fruit and vegetables, total energy intake, and hypercholesterolemia at baseline	Fifth vs. first quintile	7
Houston et al. 2011 [38]	Health ABC study USA	CVD events	1,941	9	70-79	M/F	MUFA	Age, gender, race, education, field centre, smoking, alcohol use, PA, BMI, total energy intake, protein intake, fibre intake, multivitamin use, supplemental vitamin E use, statin use, aspirin use, oral estrogen use, and prevalent diabetes or hypertension, fat, PUFA, trans fat, and cholesterol	Third vs. first tertile	8
Iso et al. 2001 [37]	Nurses' Health Study USA	Stroke	85,764	14	30-59	F	MUFA	Age, smoking status, time interval, BMI, alcohol intake, menopausal status and postmenopausal hormone use, vigorous exercise, usual aspirin use, multivitamin use, vitamin E use, n-3 fatty acid intake, calcium intake, and histories of hypertension, diabetes, high cholesterol levels, and total energy intake	Fifth vs. first quintile	7
Iso et al. 2003 [36]	JAP	Stroke	4,775	14	40-69	M/F	MUFA	Age, sex, quartiles of total energy intake and BMI, hypertension category, diabetes, serum total cholesterol, smoking status, ethanol intake, and menopausal status	Fourth vs. first quartile	8

Table 1 General study characteristics of the included cohort studies (Continued)

Jakobsen et al. 2004 [4]	Multinational MONItoring of trends and determinants in CARdiovascular disease I, II EU	CHD	3,686	16	30-71	M/F	MUFA	Total energy intake, energy from protein, energy from fat, energy from carbohydrates, non-dietary and dietary coronary heart disease risk factors	5% increase	8
Kouris-Blazos et al. 1999 [55]	AUS	All-cause mortality	330	4-6	>70	M/F	MUFA:SFA	Age, sex and smoking status but not ethnic origin	MUFA:SFA (1 unit)	6
Larsson et al. 2012 [48]	Swedish Mammography Cohort SWE	Stroke	34,670	10.4	49-83	F	MUFA	Age, smoking status and pack-years of smoking, education, BMI, PA, history of hypertension, history of diabetes, aspirin use, family history of myocardial infarction, intakes of alcohol, protein, and dietary fibre, cholesterol	Fifth vs. first quintile	8
Lasheras et al. 2000 [26]	SPA	All-cause mortality	161	9	65-95	M/F	MUFA:SFA	Age, sex, BMI, albumin concentration, PA, self-assessment of health, and dieting for chronic conditions	MUFA:SFA (1 unit)	6
Leosdottir et al. 2007 [28], Wallström et al. 2012 [29]	Malmö Diet and cancer Study SWE	CVD events Stroke CHD	28,098	13.5	44-73	M/F	MUFA MUFA:SFA	Age, smoking habits, alcohol consumption, socioeconomic status, marital status, PA, BMI, fibre intake, and blood pressure, total fat intake for the ratio between unsaturated and saturated fats	Fifth vs. first quintile Fourth vs. first quartile	9
Levitan et al. 2013 [57]	Women's Health Initiative trial and observational study USA	Heart Failure	68,132 (WHI) 93,676 (WHI-OS)	4.6	50-79	F	MUFA:SFA	Age at heart failure hospitalization, total energy intake, race/ethnicity, education, income, married, current smoking, total exercise, physical function, use of off-study postmenopausal hormone therapy, Women's Health Initiative (WHI) study arm, systolic blood pressure, diastolic blood pressure, use of diuretics, β -blockers, and angiotensin converting enzyme inhibitors or angiotensin receptor blockers, BMI, and history of high cholesterol, high blood pressure, diabetes mellitus, myocardial infarction, coronary revascularization, and atrial fibrillation	Fourth vs. first quartile	8
Martinez-Gonzalez et al. 2011 [45]	Seguimiento University of Navarra SPA	All-cause mortality	15,535	6.8	University graduates (mean: 38)	M/F	MUFA:SFA	Age, years of university of education, BMI, smoking, PA, hours per day spent watching television, history of depression, baseline hypertension, baseline hypercholesterolemia, total energy intake, egg consumption, potato consumption, and adoption of special diets	$\geq$ median vs. < median	8
Martinez-Gonzalez et al. 2009	Seguimiento University of Navarra SPA	CVD CHD	13,609	4.9	University graduates (mean: 38)	M/F	MUFA:SFA	Age, sex, family history of coronary heart disease, total energy intake, PA, smoking, BMI, diabetes at baseline, use of aspirin, history of hypertension and history of hypercholesterolemia	$\geq$ median (W: ≥ 1.24 , M: ≥ 1.19) vs. < median	8

Table 1 General study characteristics of the included cohort studies (Continued)

Misirli et al. 2012 [52]	European Prospective Into Cancer and Nutrition GRE	Stroke Incidence Stroke Mortality	23,601	10.6	20-87	M/F	MUFA Olive Oil	Age, sex, education, smoking status, BMI, PA, hypertension, diabetes, and total energy intake.	Olive oil (23 g/d) MUFA (18 g/d)	9
Nagata et al. 2012 [31]	Takayama study JAP	All-cause mortality CVD mortality Cancer mortality	28,356	16	≥35	M/F	MUFA	Age, non-alcohol energy, and protein expressed as percentage of non-alcohol energy and was additionally adjusted for fat subtypes expressed as percentage of non-alcohol energy as appropriate, height, BMI, PA, smoking status, alcohol intake, education, marital status, menopausal status, histories of diabetes and hypertension, and intakes of fruits, vegetables, and dietary fibre	Fifth vs. first quintile	8
Oh et al. 2005 [33]	Nurses' Health Study USA	CHD	78,778	20	30-55	F	MUFA	Age, BMI, smoking, alcohol intake, parental history of myocardial infarction, history of hypertension, menopausal status and hormone use, aspirin use, multivitamin use, vitamin E supplement use, PA, energy, protein, cholesterol intake, saturated, polyunsaturated, and trans-fat; a-linolenic acid; marine n-3 fatty acids; cereal fiber; and fruits and vegetables	Fifth vs. first quintile	7
Pietinen et al. 1997 [53]	Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study USA	CVD events CVD mortality	21,930	6.1	50-69	M	MUFA Oleic acid	Age, smoking, BMI, blood pressure, energy intake, alcohol, education, PA	Fifth vs. first quintile	7
Posner et al. 1991 [46]	Framingham Study USA	CHD	813	16	45-65	M	MUFA	Energy, serum cholesterol levels, PA, systolic blood pressure, left ventricular hypertrophy, cigarette smoking, glucose intolerance, and metropolitan relative weight	For recommended vs. actual intake	9
Sauvaget et al. 2004 [35]	JAP	Stroke	3,731	14	35-89	M/F	MUFA	Age, sex, adjusted for radiation dose, city, BMI, smoking status, alcohol habits, and medical history of hypertension and diabetes	Third vs. first tertile	8
Samieri et al. 2011 [10]	Three city study FRA	Stroke	7,625	5.25	≥65	M/F	Olive oil	Age, sex, education, smoking status, BMI, PA, hypertension, diabetes, and total energy intake	Third vs. first tertile	8
Solfrizzi et al. 2005 [51]	Italian Longitudinal Study on Aging ITA	All-cause mortality	278	8.5	65-84	F	MUFA MUFA:SFA	Age, sex, waist-hip ratio, smoking status, Charlson co-morbidity index, and total energy intake	Fourth vs. first quartile	7
Tognon et al. 2011 [24]	DEN	All-cause mortality	1,037	8.5	70	M/F	MUFA:SFA	Gender, BMI, waist circumference, PA, smoking status, marital status and education	≥ median vs. < median	7

Table 1 General study characteristics of the included cohort studies (Continued)

Trichopoulou et al. 2005 [23]	European Prospective Into Cancer and Nutrition Elderly EU	All-cause mortality	74,607	7.4	>60	M/F	MUFA MUFA:SFA	Age, sex, diabetes mellitus at baseline, waist to hip ratio, BMI, educational achievement, smoking status, PA at occupation, PA score at leisure, alcohol intake, and total energy intake	MUFA (12 g) MUFA:SFA (0.4)	8
Trichopoulou et al. 2009 [20]	European Prospective Into Cancer and Nutrition GRE	All-cause mortality	23,349	8.5	20-86	M/F	MUFA:SFA	Age, sex, education, smoking status, waist-to-hip ratio, BMI, MET score, and total energy intake	≥ median vs. < median	9
Trichopoulou et al. 1995 [22]	GRE	All-cause mortality	182	4-5	>70	M/F	MUFA:SFA	Age, sex, and smoking status	MUFA:SFA (1 unit)	7
Trichopoulou et al. 2003 [21]	European Prospective Into Cancer and Nutrition GRE	All-cause mortality	22,043	3.7	20-86	M/F	MUFA Olive oil MUFA:SFA	Age, sex, waist-to-hip ratio, energy-expenditure score, years of education, smoking status, BMI, and total energy intake	MUFA (15 g/d) Increment olive oil (20 g) MUFA:SFA (0.5)	9
Van den Brandt et al. 2011 [27]	Netherlands cohort study NED	All-cause mortality	120,852	10	55-69	M/F	MUFA:SFA	Age, cigarette smoking status, number of cigarettes smoked per day, years of smoking, BMI, non-occupational PA, history of hypertension, highest level of education, and energy intake	Fourth vs. first quartile	9
Yaemsiri et al. 2012 [39]	Women's Health initiative observational study USA	Stroke	87,025	7.6	50-79	F	MUFA	Age, race, education, family income, total metabolic equivalent task hours per week, alcohol intake, history of CHD, history of atrial fibrillation, history of diabetes, aspirin use, use of antihypertensive medication, use of cholesterol-lowering medication, BMI, systolic blood pressure, and total energy intake, dietary vitamin E, fruits and vegetable intake, fibre	Fifth vs. first quintile	8
Xu et al. 2006 [41]	Strong Heart Study USA	CHD CHD mortality	2,938	7.2	47-79	M/F	MUFA	Age, sex, energy, study centre, diabetes status, BMI, HDL, LDL, triacylglycerol, smoking, alcohol consumption, hypertension, percentage of energy from protein, and total energy intake	Fourth vs. first quartile	9
Wakai et al. 2014 [30]	Japan Collaborative Cohort Study JAP	All-cause mortality CVD mortality	58,672	19.3	40-79	M/F	MUFA	Age, area, education, smoking, alcohol consumption, BMI, sleep duration, walking, consumption of vegetables and fruit, and total energy intake	Fifth vs. first quintile	8

BMI Body Mass Index, *CHD* coronary heart disease, *CVD* cardiovascular disease, *DEN* Denmark, *EDI* Elderly Diet Index, *EU* European Union, *FRA* France, *GBR* Great Britain, *HDI* Healthy Diet Index, *ISR* Israel, *ITA* Italy, *JAP* Japan, *MET* metabolic equivalent of task, *MUFA* monounsaturated fatty acids, *NED* The Netherlands, *PA* physical activity, *SFA* saturated fatty acids, *SPA* Spain, *SWE* Sweden, *WHI* Women's Health Initiative, *USA* United States of America.

Table 2 Relative risk for all-cause mortality, cardiovascular mortality, combined cardiovascular events, stroke, and coronary heart disease (with 95% confidence intervals) comparing the top versus bottom third of MUFA, MUFA:SFA ratio, olive oil, and oleic acid

Outcome	No studies	MUFA source	Relative risk	95% CI	p-value	I ² (%) ^a 95% CI
All-cause mortality	17	All MUFA combined	0.89	0.83 to 0.96	0.001	64 42 to 78
	5	MUFA	1.00	0.93 to 1.08	0.93	23
	10	MUFA:SFA	0.90	0.82 to 1.00	0.04	59
	5	Olive oil	0.77	0.71 to 0.84	<0.00001	0
	14	All MUFA combined	0.88	0.80 to 0.96	0.004	50 15 to 71
Cardiovascular mortality	8	MUFA	0.96	0.89 to 1.04	0.36	7
	4	MUFA:SFA	0.91	0.83 to 0.99	0.04	0
	5	Olive oil	0.70	0.48 to 1.03	0.07	71
	1	Oleic acid	0.81	0.66 to 0.99	0.04	n.a.
	30	All MUFA combined	0.91	0.86 to 0.96	0.001	58 38 to 71
Combined cardiovascular events	20	MUFA	0.95	0.89 to 1.02	0.14	52
	6	MUFA:SFA	0.93	0.86 to 1.01	0.07	0
	7	Olive oil	0.72	0.57 to 0.91	0.007	75
	1	Oleic acid	0.87	0.76 to 1.00	0.04	n.a.
	11	All MUFA combined	0.83	0.71 to 0.97	0.02	70 46 to 84
Stroke	9	MUFA	0.85	0.72 to 1.01	0.07	65
	1	MUFA:SFA	1.18	0.91 to 1.53	0.21	n.a.
	2	Olive oil	0.60	0.47 to 0.77	<0.00001	0
	15	All MUFA combined	0.96	0.90 to 1.01	0.13	41 0 to 66
Coronary heart disease	9	MUFA	0.99	0.93 to 1.06	0.76	29
	4	MUFA:SFA	0.94	0.86 to 1.02	0.14	0
	4	Olive oil	0.80	0.57 to 1.14	0.22	77
	1	Oleic acid	0.87	0.76 to 1.00	0.04	n.a.

^aI² inconsistency, percentage of variation across studies due to heterogeneity.
MUFA monounsaturated fatty acids, n.a. not applicable, SFA saturated fatty acids.

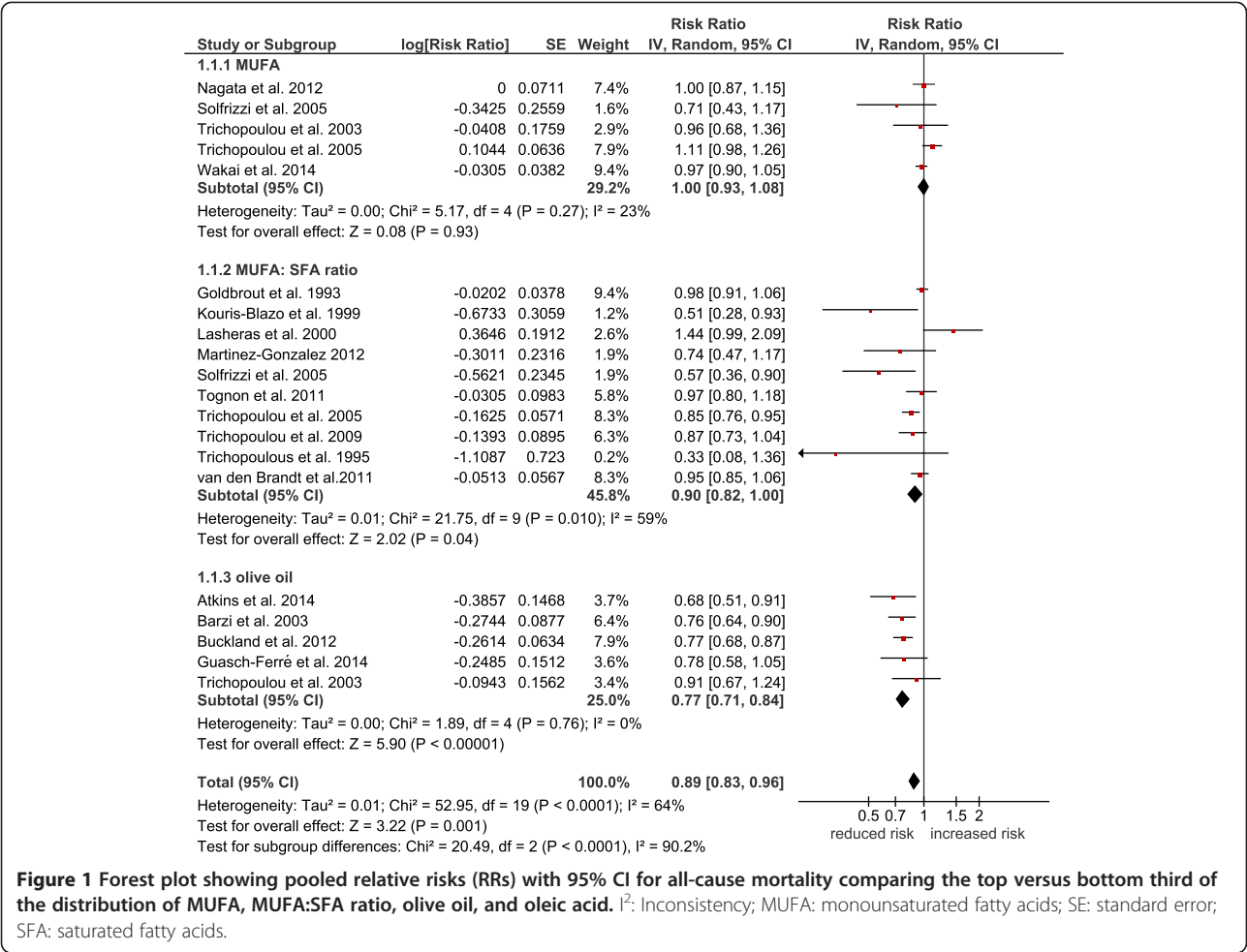
S2, S3, S4 and S5). It remains possible that small studies with inconclusive results have not been published or failed to do so.

Discussion

In the present meta-analysis, comparison of the top versus the bottom third of combined MUFA subgroups (MUFA, olive oil, oleic acid, and MUFA:SFA) was associated with reduced risk of all-cause mortality (11%), cardiovascular mortality (12%), combined cardiovascular events (9%), and stroke (17%). In the ensuing subgroup analyses, this significant correlation could only be observed between higher intakes of olive oil and reduced risk of all-cause mortality, cardiovascular events, and stroke, respectively. In contrast, monounsaturated fatty

acids of mixed animal and plant origin did not result in any significant effects with respect to these outcome parameters. Thus, it seems possible that olive oil represents the crucial factor of influence for the protective health effects observed in the primary analysis. However, one has to keep in mind the limitations of the present systematic review and meta-analysis summarized at the end of this section, especially the fact that the specific sources of MUFA have not been indicated in every study.

In order to properly evaluate the potential beneficial or detrimental effects of MUFA with respect to cardiovascular diseases, it seems of importance to consider the source of food providing these fatty acids. In the Nurses' Health Study, MUFA intake was highly correlated with



SFA intake (correlation coefficient of 0.81) but only moderately correlated with intakes of PUFA (correlation coefficient of 0.30), suggesting that fat was primarily of animal origin [58]. In the different EPIC cohorts, MUFA intakes ranged between approximately 10% of daily total energy consumption (TEC) in The Netherlands and ~20% of TEC in Greece. In general, intake of MUFA was higher in southern European countries as compared central or northern cohorts. However, another distinguishing feature seems to be the predominant source of MUFA in the respective cohorts. In Greece, Spain, and Italy, fat of plant origin (mainly olive oil) provided up to 64% of MUFA intake, whereas in most other EPIC centers, the main contributors to total MUFA intake were meat and meat products, added fats, and dairy products [8]. This might also provide an explanation for the somewhat mixed results provided by systematic reviews and meta-analyses in the past. Thus, a diet rich in MUFA was found to have beneficial effects on a broad range of CVD risk factors, not only in the primary prevention of CVD [1,59-61]. On the other hand, no association between total and individual MUFA and CHD was reported in a meta-analysis of studies assessing both dietary intake and circulating fatty acid composition, [7] while a meta-analysis of observational studies suggested that replacing SFAs with PUFAs might have a greater benefit than replacement of SFAs by MUFA [4]. There is some evidence drawn from prospective studies of an adverse association between MUFA and coronary events, but this correlation might be influenced by high amounts of MUFA of animal origin [4].

A number of *in-vivo* and *in-vitro* studies examined the health effects of extra virgin olive oil, the potential “Unique Selling Proposition” of a genuine Mediterranean diet. Thus, the Di@bet.es study demonstrated that individuals who consumed olive oil had a significantly lower risk of developing obesity, impaired glucose metabolism, hypertriglyceridemia, and lower HDL cholesterol levels as compared to a group consuming sunflower oil [62]. In addition, results from experimental studies provide evidence that olive oil consumption improves several CHD risk factors [63,64]. The PREDIMED dietary intervention trial aimed a intake of 50 g/d or more of extra virgin olive oil observed a significant risk reduction of both combined cardiovascular events as well as primary

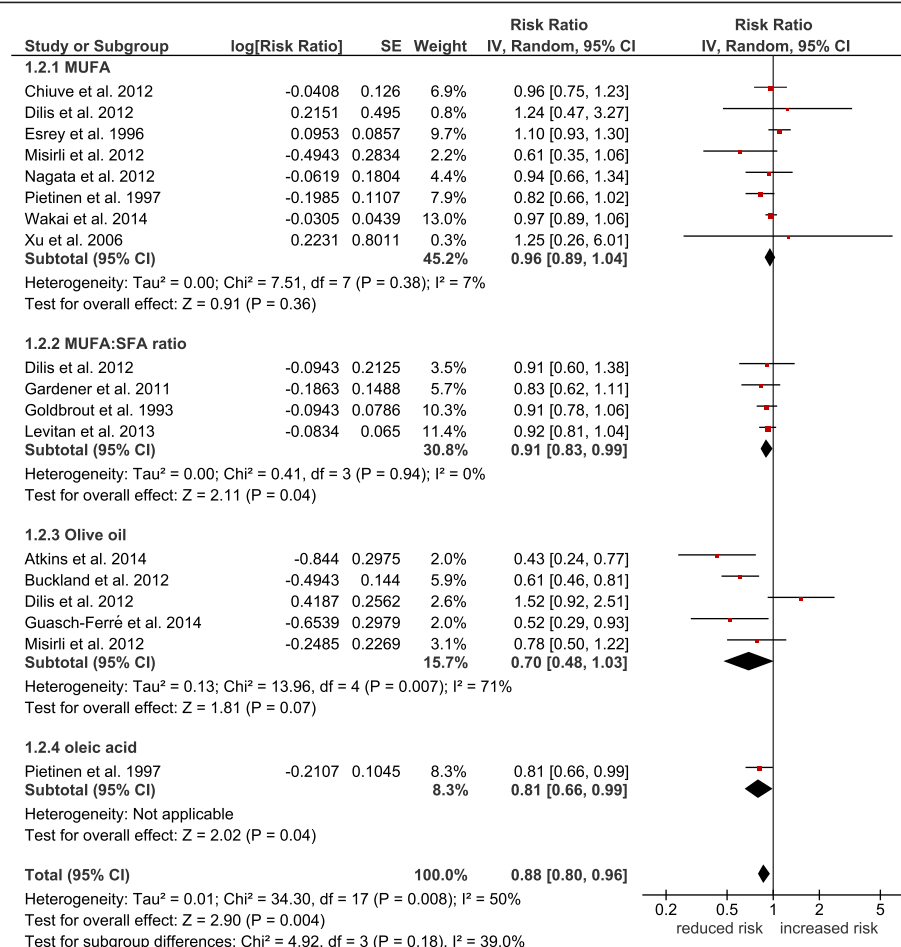


Figure 2 Forest plot showing pooled relative risks (RRs) with 95% CI for cardiovascular mortality comparing the top versus bottom third of the distribution of MUFA, MUFA:SFA ratio, olive oil, and oleic acid. I^2 : Inconsistency; MUFA: monounsaturated fatty acids; SE: standard error; SFA: saturated fatty acids.

stroke, but not of CHD, indicating a consistency with the results of the present meta-analyses of cohort studies [9]. In a long-term intervention trial by Esposito et al., a higher regression in as well as a lower rate of progression of the intima-media thickness of the carotid artery was found in the group adopting a Mediterranean diet as compared to a low-fat diet reference arm [65].

Apart from oleic acid, olive oil contains a number of bioactive compounds such as polyphenols which are especially prominent in virgin and extra-virgin olive oil, but not in refined olive oil [64,66]. A key olive oil polyphenol is oleuropein (a compound that generates tyrosol and hydroxytyrosol), which accounts for approximately 80% of olive oil phenolic content and is a potent scavenger of superoxide radicals and inhibits LDL oxidation [67,68]. There is a causal link between oxidative stress, inflammation, endothelial dysfunction, and CVD/CHD [69]. A meta-analysis of intervention trials provide evidence that an MD decreases inflammation and improves endothelial function [70]. When focusing on virgin olive

oil consumption, the inverse correlation between olive oil and CHD risk found in the present meta-analysis is consistent with the fact that olive oil is not just a supplier of MUFA but of other biologically active components as well.

Several limitations should be taken into account when interpreting the results of the present meta-analysis. MUFA coexist with SFA in several food sources. In addition, cis- and trans-isomers of MUFAs were sometimes categorized together in cohort studies. Furthermore, moderate to substantial heterogeneity could be observed in the present meta-analysis. Potential sources of heterogeneity include combining MUFA/olive oil/oleic acid/MUFA:SFA ratio in the same analysis, heterogeneous risk estimates, heterogeneous populations/ages/gender, sample sizes as well as follow-up periods of the included studies. No unpublished data were considered for the present meta-analysis, and it cannot be excluded that these results may influence the effect size estimates. Examination of funnel plots showed little to moderate asymmetry

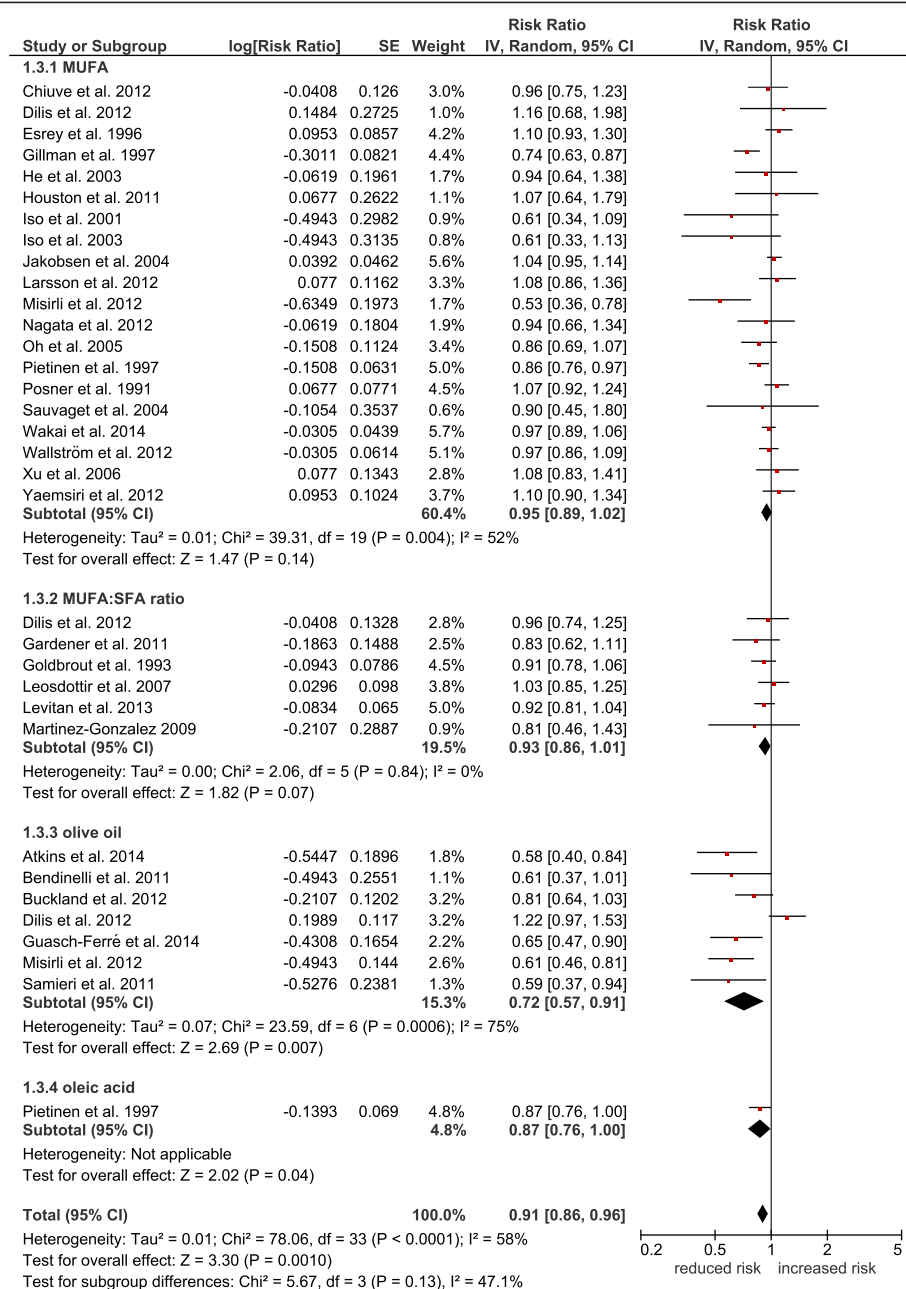


Figure 3 Forest plot showing pooled relative risks (RRs) with 95% CI for combined cardiovascular events comparing the top versus bottom third of the distribution of MUFA, MUFA:SFA ratio, olive oil, and oleic acid. I^2 : Inconsistency; MUFA: monounsaturated fatty acids; SE: standard error; SFA: saturated fatty acids.

suggesting that publication bias cannot be completely excluded as a confounder of the present meta-analysis (e.g. it remains possible that small studies yielding inconclusive data have not been published). In addition, the specific food sources of MUFA could not always be identified, limiting the validity of any general recommendation towards MUFAs of plant origin (it is most likely olive oil, but it might be other types of food as well, e.g. nuts, canola oil or a specific variety of sunflower oil). Conversely, it might

be that results from studies using mixed sources of MUFA might be biased by non-identified olive oil, making MUFA appear to be beneficial in general when some sources are not. Furthermore, observational studies including cohort studies assessing outcome events affected by nutrition should be interpreted with caution, since reliance on nutritional assessment methods with validity and reliability is lower when compared to randomized controlled trials.

However, the present study has some complementary strengths as well. Compared to cohort studies, dietary intervention trials are often limited by lack of double blinding, non-compliance, cross-over, and high drop-out rates. Therefore, well-designed analyses in prospective cohort studies could also provide important evidence with respect to long-term clinical outcomes. Another strength of this work is the inclusion of an overall population >800,000 subjects. To the best of our knowledge, this represents the most comprehensive summary of the evidence on MUFA, olive oil, MUFA:SFA on hard clinical outcome parameters.

Conclusion

The results of the present meta-analysis indicate an overall risk reduction of all-cause mortality (11%), cardiovascular mortality (12%), cardiovascular events (9%), and stroke (17%) when comparing the top vs. bottom thirds of a combination of MUFA, olive oil, oleic acid, and MUFA:SFA ratio. Monounsaturated fat of mixed animal and vegetable sources per se did not yield any significant effects on these outcome parameters. Subgroup analysis indicated that only olive oil (the primary monounsaturated fat source in south European countries) is associated with a significant risk reduction for several outcomes. These data provide evidence that the source and origin of MUFA within a specific diet should be taken into account in order to evaluate the potential benefits of this type of fatty acids. Further studies are required evaluating specific food sources of MUFA and risk of all-cause mortality and CVD events.

Additional file

Additional file 1: Detailed search strategy; Figure S1-S5: Funnel Plots.

Competing interests

Both authors declare that they have no competing interest.

Authors' contributions

LS and GH conducted the data analysis, interpretation of results, manuscript drafting, and finalizing manuscript. Both authors read and approved the final manuscript.

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Review

Monounsaturated Fatty Acids and Risk of Cardiovascular Disease: Synopsis of the Evidence Available from Systematic Reviews and Meta-Analyses

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Abstract: No dietary recommendations for monounsaturated fatty acids (MUFA) are given by the National Institute of Medicine, the United States Department of Agriculture, European Food and Safety Authority and the American Diabetes Association. In contrast, the Academy of Nutrition and Dietetics, and the Canadian Dietetic Association both promote <25% MUFA of daily total energy consumption, while the American Heart Association sets a limit of 20% MUFA in their respective guidelines. The present review summarizes systematic reviews and meta-analyses of randomized controlled trials and cohort studies investigating the effects of MUFA on cardiovascular and diabetic risk factors, cardiovascular events and cardiovascular death. Electronic database Medline was searched for systematic reviews and meta-analyses using “monounsaturated fatty acids”, “monounsaturated fat”, and “dietary fat” as search terms with no restriction to calendar date or language. Reference lists and clinical guidelines were searched as well. Sixteen relevant papers were identified. Several studies indicated an increase of HDL-cholesterol and a corresponding decrease in triacylglycerols following a MUFA-rich diet. The effects on total and LDL-cholesterol appeared not consistent, but no detrimental effects on blood lipids were observed. Values for systolic and diastolic blood pressure were found to be reduced both during short- and long-term protocols using high amounts of MUFA as compared to low-MUFA diets. In type 2 diabetic subjects, MUFA exerted a hypoglycemic effect and reduced glycosylated hemoglobin in the long term. Data from meta-analyses exploring evidence from long-term prospective cohort studies provide ambiguous results with respect to the effects of MUFA on risk of coronary heart disease (CHD). One

meta-analysis reported an increase in CHD events, however, most meta-analyses observed a lesser number of cases in participants subjected to a high-MUFA protocol. Although no detrimental side effects of MUFA-rich diets were reported in the literature, there still is no unanimous rationale for MUFA recommendations in a therapeutic regimen. Additional long-term intervention studies are required to characterized efficacy and effectiveness of recommending MUFA-rich diet among general and clinical populations.

Keywords: monounsaturated fatty acids; cardiovascular disease; coronary heart disease; meta-analysis; systematic review; dietary fat

1. Monounsaturated Fatty Acids

Monounsaturated fatty acids (MUFA) are chemically classified as fatty acids containing a single double bond (in contrast to polyunsaturated fatty acids (PUFA) containing two or more double bonds and saturated fatty acids (SFA) without double bonds). In the *cis*-configuration, the hydrogen atoms are on the same side as the double bond, whereas in *trans*-configuration the hydrogen atoms and the double bond are present on opposite sides. The *cis*-isomers are the predominant form of MUFA in food sources. The most common *cis*-configured MUFA in daily nutrition is oleic acid (18:1 *n*-9), followed by palmitoleic acid (16:1 *n*-7), and vaccenic acid (18:1 *n*-7). Moreover, oleic acid represents the topmost MUFA provided in the diet (~90% of all MUFAs) [1]. The major *trans*-configured MUFA is elaidic acid (*trans* 18:1 *n*-9). Some MUFA—such as myristoleic (14:1 *n*-5), gondoic (20:1 *n*-9), erucic (22:1 *n*-9) and nervonic (24:1 *n*-9) acid—are synthesized in minor concentrations endogenously using other MUFAs as precursors (see Table 1 for a summary of different types of MUFA). Various sources for MUFA in food are given in Table 2 (for comparison, PUFA and SFA contents are given as well). The most frequently consumed MUFA rich dietary oils are canola and olive oil. Furthermore, over the last decade commercial production of high oleic acid modified dietary oils with improved stability for the use in food processing has been markedly increased in order to replace dietary oils rich in SFA and *trans* fatty acids [2]. It should be recognized that in some populations, MUFAs are provided in higher amounts in the form of erucic acid (C22:1 *n*-9), e.g., found in culinary oils derived from some *Brassica* spp. such as rapeseed and mustard seed [3]. It is therefore not surprising that due to their widespread occurrence in oils nuts, seeds, fruits and meat, the predominant source of MUFA is largely depending on individual dietary habits. Like other fatty acids, MUFA are almost completely absorbed by the intestine and are oxidized for energy production, converted into other fatty acids, or incorporated into tissue lipids.

Table 1. Selected monounsaturated fatty acids.

C-Atoms: Double Bonds	Scientific Name of Acid	Molecular Formula	Chemical Name
11:1	Undecylenic	C ₁₀ H ₁₉ COOH	<i>cis</i> -10-undecenoic acid
14:1	Myristoleic	C ₁₃ H ₂₅ COOH	<i>cis</i> -9-tetradecenoic acid
16:1	Palmitoleic	C ₁₅ H ₂₉ COOH	<i>cis</i> -9-hexadecenoic acid
16:1	Palmitelaidic	C ₁₅ H ₂₉ COOH	<i>trans</i> -9-hexadecenoic acid
16:1	/	C ₁₅ H ₂₉ COOH	<i>cis</i> -7-hexadecenoic

Table 1. Cont.

18:1	Petroselinic	C ₁₇ H ₃₃ COOH	<i>cis</i> -6-octadecenoic acid
18:1	Oleic	C ₁₇ H ₃₃ COOH	<i>cis</i> -9-octadecenoic acid
18:1	Elaidic	C ₁₇ H ₃₃ COOH	<i>trans</i> -9-octadecenoic acid
18:1	Vaccenic	C ₁₇ H ₃₃ COOH	<i>cis</i> -11-octadecenoic acid
20:1	Gondoleic	C ₁₉ H ₃₇ COOH	<i>cis</i> -9-eicosenoic acid
20:1	Gondolic	C ₁₉ H ₃₇ COOH	<i>cis</i> -11-eicosenoic acid
22:1	Cetoleic	C ₂₁ H ₄₁ COOH	<i>cis</i> -11-docosenoic acid
22:1	Erucic	C ₂₁ H ₄₁ COOH	<i>cis</i> -13-docosenoic acid
24:1	Nervonic	C ₂₃ H ₄₅ COOH	<i>cis</i> -15-tetracosanoic acid

Table 2. Fatty acid content of different oils, nuts, fruits, seeds and animal products.

Oils	MUFA, %	PUFA, %	SFA, %
Olive oil	73	10.5	14
Coconut oil	6	2	86
Soybean oil	23	58	16
Peanut oil	46	32	17
Sesame oil	40	42	14
Sunflower oil (linoleic acid <60%)	45	40	10
High-oleic safflower oil	72	13	7.5
Sunflower oils (linoleic acid >70%)	14	75	6
Walnut oil	23	63	9
Almond oil	70	17	8
Hazelnut oil	78	10	7
Avocado oil	71	13	12
Canola oil	63	28	7
Mustard oil	59	21	12
High oleic sunflower	84	4	10
Herring oil	57	16	21
Fish oil, cold liver	47	23	23
Flaxseed oil, cold press	18	68	9
Corn and canola oil	58	29	8
High oleic sunflower	84	4	10
Hazelnut oil	78	10	7
Olive oil	73	10.5	14
High-oleic safflower oil	72	13	7.5
Avocado oil	71	13	12
Almond oil	70	17	8
Canola oil	63	28	7
Mustard oil	59	21	12
Corn and canola oil	58	29	8
Herring oil	57	16	21
Fish oil, cold liver	47	23	23
Peanut Oil	46	32	17
Sunflower Oil (linoleic acid <60%)	45	40	10
Sesame Oil	40	42	14

Table 2. *Cont.*

Soybean oil	23	58	16
Walnut oil	23	63	9
Flaxseed oil, cold press	18	68	9
Sunflower oils (linoleic acid >70%)	14	75	6
Coconut oil	6	2	86
Nuts and Seeds	MUFA, %	PUFA, %	SFA, %
Macademia	59	12	2
Hazelnut	46	8	4
Pecanut	41	22	6
Almonds	31	11	4
cashew nuts, dry roasted	27	7	9
Pistachio nuts	24	14	5
Sunflower seed kernels, dried	19	23	4
Sesame, whole, roasted and toasted	18	21	7
Walnuts	15	35	3
Flaxseed	8	29	4
Safflower kernels, dried	5	28	4
Products of Animal Origin	MUFA, %	PUFA, %	SFA, %
Butter, salted	21	3	51
Cheese, cheddar	9	1	21
Pork, ham	8.3	2	6.5
Mackerel	5.4	3.3	3.2
Beef, steak	4.5	0.4	4.3
Egg	3.6	2	3
Salmon	2.1	2.5	0.9
Milk, 3.7% fat	1	0.1	2.2
Chicken	0.9	0.75	0.8

MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid [4].

2. Guidelines

2.1. General Nutrition Guidelines

Table 3 summarizes MUFA recommendations of national and international authorities and organizations.

Table 3. National and international MUFA recommendations for healthy adults and patients with diabetes.

Authority/Society	MUFA (% of TEC)	Target Group/Remarks	References
<i>American Heart Association</i>	<20	Healthy adults	[5]
<i>Academy of Nutrition and Dietetics/Canadian Dietetic Association</i>	<25	Healthy adults	[6]
<i>Dutch Dietary Guidelines</i>	8–38	Healthy adults	[7]
<i>European Food Safety Authority</i>	No specific recommendations	Upper limit for obese: 25% of TEC Healthy adults	[8]

Table 3. Cont.

<i>Italian Society of Human Nutrition</i>	No specific recommendations	Healthy adults	[9]
<i>Joint Committees of Germany, Austria, and Switzerland</i>	10	Healthy adults	[10]
<i>National Cholesterol Educational Program III</i>	<20	Healthy adults	[11]
<i>National Institute of Medicine</i>	No specific recommendations	Healthy adults	[12]
<i>Nordic Nutrition Dietary Guidelines</i>	10–15	Healthy adults	[13]
<i>Nutritional Recommendations for the French Population</i>	20	Healthy adults Including pregnant and lactating women	[14]
<i>UK COMA Committee</i>	12	Healthy adults	[15]
<i>US Department of Agriculture</i>	No specific recommendations	Healthy adults	[16]
<i>World Health Organization/Food Agriculture Organization</i>	15–20	Healthy adults Adjusted to total fat intake	[3]
<i>American Association of Clinical Endocrinologists</i>	No specific recommendations	Diabetic patients	[17]
<i>American Diabetes Association</i>	No specific recommendations	Diabetic patients Initial recommendation: 10%–20% of TEC	[18,19]
<i>British Diabetes Association</i>	10–15	Diabetic patients	[20]
<i>Canadian Diabetes Association</i>	No specific recommendations	Diabetic patients Replacement of SFA by MUFA	[21]
<i>European Association for the Study of Diabetes</i>	10–20	Diabetic patients Limitation of total fat to 35% of TEC	[22]
<i>International College of Nutrition of India</i>	7	Diabetic patients	[23]

MUFA = monounsaturated fatty acids; SFA = saturated fatty acids; TEC = total energy content.

In 1999, the International Society for the Study of Fatty Acids and Lipids agreed upon a recommendation table on daily intake of fatty acids as a foundation for further discussions. Adequate intake levels for adults were specified with respect to α -linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, as well as upper limits for linoleic acid, *trans*-fatty acids, and saturated, given as % of total energy content (TEC), respectively. Given a total fat range from 15% to 40% of TEC, these recommendations included the suggestion to provide the majority of fatty acids in the form of MUFAs. However, no precise value (*i.e.*, % of TEC in the form of MUFA) was given by the panel [24]. According to the Joint FAO/WHO Expert Consultation Committee, MUFA intakes should be determined by calculating the difference: MUFA (% of TEC) = total fat (% of TEC) – SFA (% of TEC) – PUFA (% of TEC) – TFA (% of TEC). Accordingly, MUFA intakes (% of TEC) will range with respect to the total fat and fatty acid composition of the diet [3]. Based upon 13 peer-reviewed background papers dealing with fats and fatty acids in human nutrition, the Joint FAO/WHO Expert Consultation Committee concluded that replacement of carbohydrates by MUFA beneficially increases HDL-cholesterol, while the substitution of SFA with MUFA exerts favorable effects on LDL-cholesterol and the ratio of total cholesterol to HDL-cholesterol [3]. In their position on dietary fatty acids of

2007, the American and Canadian Dietetic Association suggested a high maximum quota of MUFA, *i.e.*, <25% of TEC [6]. Less than 20% of TEC should be consumed in the form of MUFA as recommended by the American Heart Association (AHA) in 2006, which is interesting with respect to the fact that the corresponding value was only <15% in the year 2000-statement of the AHA [5,25]. The National Cholesterol Education Program III suggested that <20% of TEC should be consumed in the form of MUFA [11]. In their Dietary Guidelines for Americans, edition 2010 [16], the United States Department of Agriculture (USDA) gives no specific recommendations for MUFA [16]. In addition, the National Institute of Medicine did not mention any specific recommendations for MUFA. In their statement, they concluded that “*n-9 cis* Monounsaturated fatty acids are synthesized by the body and have no known independent beneficial role in human health and are not required in the diet.” Therefore, neither an Adequate Intake nor a Recommended Daily Allowance was set. Since there is insufficient evidence for an Upper Level as well, the Dietary Reference Intakes did not consider MUFA at all [12]. In accordance with these proceedings and with a similar rationale, the European Food and Safety Authority (EFSA) skipped MUFA in their scientific opinions on dietary reference values for fat [8]. On a national level, the recommendations given in European countries are far from being conclusive. The Italian Society of Human Nutrition did not list any specific references for MUFA [9]. The Joint Committees of Germany, Austria, and Switzerland stated that MUFA consumption should be 10% of TEC, albeit with higher intakes being acceptable [10]. The Nordic Nutrition Recommendations agreed on 10%–15% of TEC in the form of MUFA [13]. The particulars of the Dutch Dietary Guidelines proposed a limit of 38% of TEC in the form of MUFA and PUFA for people with optimal body weight, whereas overweight and obese people should be more restrictive and limit their daily energy uptake in the form of MUFA/PUFA to 28% [7]. The UK COMA Committee advocated that MUFA (in the form of oleic acid) should provide an average of 12% of TEC [15]. The Nutritional Recommendations for the French Population promoted an intake of MUFA up to 20% of TEC for adults including pregnant and lactating women. It was emphasized that the neutrality of oleic acid represents a benefit and that its consumption was justified [14].

2.2. Specific Guidelines for the Prevention and Treatment of Diabetes

On closer examination, the MUFA recommendations of the American Diabetes Association (ADA) evolved in a “downhill” fashion. In 2002, a consumption of 10%–20% of TEC in the form of MUFA was proposed [18]. Two years later, a carbohydrate plus MUFA intake of 60%–70% of TEC was regarded as an evidence-based nutrition principle for the prevention and treatment of diabetes [26]. In 2008, the ADA’s position statement did not offer a specific value for MUFA as a preventive or therapeutic tool any longer [19]. Correspondingly, the American Association of Clinical Endocrinologists excluded MUFA in their medical guidelines for the management of diabetes [17]. However, the Canadian Diabetes Association suggested frequently replacing SFA with MUFA for a successful nutritional management of diabetes mellitus [21]. Likewise, the Joslin Diabetes Center recommended the consumption of MUFA, again without any specific reference values [27]. The Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes stated that MUFA should provide 10%–20% of TEC with total fat to be limited to 35% of TEC [22]. The British Diabetes Association, probably referring to the 2004 nutrition principles of the ADA, recommended

a daily amount of 60%–70% of TEC in the form of carbohydrates and MUFA, with MUFA values specified separately to aim at 10%–15% of TEC [20]. In Japan, no specific quota of MUFA is given in as a nutritional reference, while other Asian nations like India allow for up to 7% of TEC in the form of MUFA [23,28]. In South Africa, the corresponding authorities recommended <13% MUFA for diabetic subjects [29].

3. Risk Factors for Diabetes and Cardiovascular Disease

The National Cholesterol Education Program guidelines have outlined risk factors that increase CHD risk over a 10 year period. Elevated LDL-cholesterol (>100 mg/dL) remains the strongest primary factor in predicting CHD and therefore is a primary target of therapy [11]. However, as circulating triacylglycerols (TG) and HDL-cholesterol concentrations are critical risk factors in metabolic syndrome, the TC:HDL-cholesterol ratio has been expressed as a more valuable marker in determining CHD risk [30]. Summing-up, elevated levels of TC, LDL-cholesterol and TG as well low levels of HDL-cholesterol are evidence-based risk factors of CVD [31–33]. High levels of blood pressure are also associated with an increased mortality risk [34]. In addition, the Emerging Risk Factor Collaboration indicated FG levels >100 mg/dL as a predictor of mortality [35]. The Framingham Heart Study showed that impaired fasting glucose was associated with an aggravated risk of CHD in women [36]. A meta-analysis of cohort studies including 44,158 individuals without diabetes found a significant association between glycosylated hemoglobin (HbA1c) and cardiovascular events as well as death [37]. In another meta-analysis of observational studies, it was concluded that chronic hyperglycemia is associated with an increased risk of CVD in patients with type 2 diabetes mellitus (T2D) [38]. Among women, high-sensitive C-reactive protein (CRP) was the strongest predictor of CVD, accompanied by TC, LDL-cholesterol, TC:HDL-cholesterol, and Apo B 100 [39]. A recent meta-analysis indicated that Apo B is a more accurate marker of cardiovascular risk as compared to non-HDL-cholesterol (=TC-HDL-cholesterol), while the latter is still more accurate in comparison to LDL-cholesterol [40]. Strong associations between low serum HDL-cholesterol/high serum LDL-cholesterol and the onset of abdominal aortic aneurysms prove the continuous validity of both markers as predictive risk factors [41]. A collaborative analysis of individual data from 36 prospective studies involving more than 126,000 individuals, has demonstrated that circulating Lp(a) concentrations are correlated with an increased incidence of CHD and stroke independent from several conventional risk factors (including TC) [42].

4. Methods

4.1. Data Sources and Search Strategy

Electronic database MEDLINE (between 1966 and November 2012) was searched for systematic review and meta-analysis using following search terms “monounsaturated fatty acids”, “monounsaturated fat” and “dietary fat” with no restriction to calendar data and language. Reference lists and relevant clinical guidelines were also searched.

4.2. Inclusion Criteria

Studies were included in this review if they met all of the following criteria: (1) systematic review/meta-analysis (quantitative analysis) including RCTs, crossover, metabolic, and observational studies; (2) intervention trials (isocaloric exchange): comparison of MUFA *vs.* carbohydrates, SFA, PUFA, and *trans*-fat; cohort studies: highest MUFA intake *vs.* lowest MUFA intake; (3) Study population: >18 years, healthy, patients with type 2 diabetes mellitus (T2D), obese, overweight; impaired glucose metabolism and cardiovascular disease (CVD); (4) outcome parameters: anthropometric outcomes, blood lipids, glycemic control parameters, blood pressure, inflammation markers and cardiovascular events/mortality.

4.3. Study Quality Assessment

Review quality was rated using a modified version of the Overview of Quality Assessment Questionnaire (OQAQ) including a bias tool [43] (Supplemental material, Table S1) as described recently [44]. Results of OQAQ assessments are summarized in Table 4. It should be noted that the analyses considered were in some cases based on overlapping sets of trials.

Table 4. Qualitative aspects of the included systematic reviews and meta-analyses.

Reference	Aim	Methods (Inclusion/Exclusion criteria)	Heterogeneity	Period	Quality Assessment
Hegsted <i>et al.</i> 1993 [45]	Overall evaluation of the rather extensive literature on the effects of dietary fatty acid composition and cholesterol on serum lipid concentration	Design: metabolic studies (appear to have been done under rather careful control in which food was prepared and fed to the subjects); field trials (diet was modified by instructions or by a combination of instructions and provision of some foods)	not analyzed	until 1991	8
Mensink <i>et al.</i> 1992 [46]	Combining results to derive equations that relate changes in the dietary fatty acid intake to changes in serum HDL-C, LDL-C, TC and TG	Design: parallel design, crossover or Latin-square; “before and after” designs that lacked a control group were excluded. Diets enriched with very-long-chain (<i>n</i> -3) PUFA were also excluded	not analyzed	1970–1991	10
Gardner <i>et al.</i> 1995 [47]	The purpose of this investigation was to address the controversy regarding a differential effect of MUFA <i>vs.</i> PUFA on serum lipids	Design: randomized trials comparing a high-mono and high-poly fat diet; similar in all respects (isoenergetic, total fat content, SFA) except for levels of monounsaturated and polyunsaturated fat intake; minimum 10 subjects on each diet arm	analyzed	1966–1994	12
Yu <i>et al.</i> 1995 [48]	Conducted to more comprehensively examine the effects of stearic acid, MUFAs, and other fatty acids on total and lipoprotein cholesterol concentrations in both men and women	Studies reported the quantity of individual SFA or stearic acid, sum of lauric, myristic and palmitic acids, and sum of MUFA and PUFA of the experimental diets. Exclusion. Liquid formula diets; diets that were specifically enriched with in <i>trans</i> isomers; diets enriched with very-long-chain PUFA; subject with familiar hypercholesterolemia	not analyzed	1970–1993	8

Table 4. Cont.

Clarke <i>et al.</i> 1997 [49]	The aim of this meta-analysis of metabolic ward studies is to provide reliable quantitative estimates of the relevance of dietary intake of fatty acids and dietary cholesterol to blood concentrations of total cholesterol and cholesterol fraction	Design: dietary intervention studies conducted under controlled conditions that ensured compliance	not analyzed	/	9
Garg 1998 [50]	Examining the effects of high carbohydrate low fat diets vs. high MUFA diets on metabolic indexes in T2D subjects	Design: randomized, crossover trials using isoenergetic, weight maintaining diets	not analyzed	/	9
Mensink <i>et al.</i> 2003 [30]	Combining results to derive equations that relate changes in the dietary fatty acid intake to changes in serum HDL-C, LDL-C, TC and TG, Apo-B and Apo A-I, TC:HDL-C	Design: parallel design, crossover or Latin-square; “before and after” designs that lacked a control group were excluded. Diets enriched with very-long-chain (<i>n</i> -3) PUFA were also excluded	not analyzed	1970– 1998	13
Shah <i>et al.</i> 2007 [46]	Comparing high carbohydrate and high- <i>cis</i> -MUFA interventions trials conducted to increase understanding of the effect of carbohydrate and <i>cis</i> -MUFA on blood pressure	Design: randomized and non-randomized intervention studies comparing the effects of high-carbohydrate diets with those of high- <i>cis</i> -MUFA diets on blood pressure (crossover or parallel design), comparison of diets isoenergetic, body weight had to remain stable	analyzed	until 2006	12
Cao <i>et al.</i> 2009 [51]	Objective was to quantify the magnitude of the changes in lipids and lipoproteins in response to a MF blood cholesterol-lowering diet rich in unsaturated fat vs. LF in subjects with and without diabetes	Design: controlled feeding with a crossover or parallel design comparing MF vs. LF diets; designed to lower blood lipids; comparisons were isoenergetic; participants maintained constant weight during study; dietary protein and cholesterol were kept constant between diets	not analyzed	1987– 2007	14
Jakobsen <i>et al.</i> 2009 [52]	Associations between energy intake from MUFA, PUFA, and carbohydrates and risk of CHD while assessing the potential effect-modifying role of sex and age	Design: cohort studies; published follow-up study with ≥ 150 incident coronary events; availability of usual dietary intake; a validation or repeatability study of the diet-assessment method used	analyzed	/	10
Kodama <i>et al.</i> 2009 [53]	To elucidate the effect of replacing dietary fat with carbohydrate on glucose and lipid parameters	Design: randomized controlled trials (crossover and parallel-group design); isoenergetic; only T2D Exclusion: T1D, diets with change in in the content or quality of carbohydrates; heterogeneity analyzed	analyzed	1966– 2007	16
Mente <i>et al.</i> 2009 [54]	Examining the association between nutrient intake, dietary components, and dietary patterns and CHD and its related clinical outcomes	Design: cohort studies; dietary pattern: higher intake level is compared with lowest intake level; <i>p</i> -values for trend, where available, were used to evaluate dose-response relationship. FFQ, food records, 24 h recalls; Bradford Hill criteria	analyzed	1950– 2007	15
Mozaffarian and Clarke 2009 [55]	Examining the effects on CHD risk of replacing partially hydrogenated formulations on other specific fats on the basis of the content of TFA, SFA, MUFA and PUFA	Design: randomized controlled trials (consumption of fatty acids on risk factors), cohort studies (association of habitual intake of fatty acids with incidence of CHD events); isocaloric replacement	not analyzed	until 2008	10

Table 4. Cont.

Skeaff and Miller 2009 [56]	The purpose of this article was to summarize the evidence from cohort studies and randomized controlled trials of the relation between dietary fat and risk of CHD	Design: cohort studies; quintiles intake of PUFA, MUFA, SFA, TFA; The dietary assessment methods used in the cohort studies included single 24 h recall, diet records, diet histories and food frequency questionnaires; For MUFA only studies included in which exposure was determined by dietary assessment because blood fatty acids are not good biomarkers of MUFA intake	analyzed	/	10
Schwingshackl <i>et al.</i> 2011 [57]	Comparing high MUFA (>12% of TEC) vs. low MUFA (≤12% MUFA of TEC) on cardiovascular risk factors	Design: randomized controlled trials, ≥6 months, isocaloric and hypocaloric diets; subgroup analysis MUFA vs. LF, PUFA, LGI, HGI, Controls	analyzed	1966– 2011	13
Schwingshackl <i>et al.</i> 2011 [58]	Comparing high MUFA (>12% of TEC) vs. low MUFA (≤12% MUFA of TEC) on glycemic control in subjects with abnormal glucose metabolism	Design: randomized controlled trials, ≥6 months, isocaloric and hypocaloric diets, subgroup analysis MUFA vs. LF, PUFA, LGI, HGI, Controls	analyzed	1966– 2011	13

Apo A I: Apolipoprotein A-I; Apo B: Apo lipoprotein B; CHD: coronary heart disease; FFQ: food frequency questionnaire; HDL-C: high-density lipoprotein cholesterol; HGI: high glycemic index; LDL-C: low-density lipoprotein cholesterol; LF: low fat; LGI: low glycemic index; MF: moderate fat; MUFA: monounsaturated fat; PUFA: polyunsaturated fat; SFA: saturated fat; T2D: type 2 diabetes subjects; TC: total cholesterol; TEC: total energy content; TFA: *trans* fat; TG: triacylglycerols.

The present review included meta-analyses of intervention trials (randomized, non-randomized and crossover trials) and cohort studies. A common problem associated with cross-over trials is that of carry-over (a type of period-by-intervention interaction), but it seems only justifiable to exclude cross-over trials from a systematic review if the design is inappropriate within the clinical context [59]. Duration of studies varied remarkably between the different meta-analyses as well as between the different within each meta-analysis. This represents a major problem especially when comparing intervention trials. Sensitivity analyses comparing short- vs. long-term studies might be used as an alternative approach to resolve this issue. Another issue associated with meta-analyses is heterogeneity of various aspects and characteristics of the study protocols, especially in nutritional intervention trials. Therefore, it is not surprising that the literature chosen for the present review varies regarding type(s) of diets used (MUFA vs. carbohydrates/PUFA/SFA/*trans* fatty acids), definitions of MUFA diets, and study population (healthy, overweight, or obese subjects, patients with T2D, abnormal glucose metabolism, or CVD). In addition, in most of the included meta-analyses differential compliance (drop outs) was not investigated. Another potential source of bias is measurement issues (especially of self-reported data, e.g., 24 h recalls, food records). Only few systematic reviews screened for the presence of publication bias by assessing the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors.

5. Evidence from Meta-Analyses

5.1. Healthy Subjects

See Table 5 summarizes the study characteristics of the meta-analyses included in this review. For a better understanding of the categorization of meta-analyses and other scientific studies, the Levels of evidence by the Scottish Intercollegiate Guidelines Network are given in Table 6 [60].

Table 5. Study characteristics of meta-analyses.

Reference	No. Studies	Statistical Method	Min. Duration	Participants	Effects of MUFA
Hegsted <i>et al.</i> 1993 [45]	<i>n</i> = 77	Multiple regression	n.d.	n.d.	↔ TC, LDL-C, HDL-C
Mensink <i>et al.</i> 1992 [61]	<i>n</i> = 28	meta-regression	14 days	682	↓ TG, HDL-C:LDL-C ↑ HDL-C ↔ TC, LDL
Gardner <i>et al.</i> 1995 ** [47]	<i>n</i> = 14	Standardized effect size	3 weeks	439	↑ TG * ↔ LDL-C, HDL-C
Yu <i>et al.</i> 1995 [48]	<i>n</i> = 18	Meta-regression analysis	n.d.	804	↓ TC, LDL-C ↑ HDL-C
Clarke <i>et al.</i> 1997 [49]	<i>n</i> = 91	Multilevel regression analysis	2 weeks	5910	↑ HDL-C ↔ TC, LDL-C
Garg 1998 [50]	<i>n</i> = 9	meta-analysis	2 weeks	133	↓ TG, TC, VLDL-C, FG ↑ HDL-C, Apo A-1 ↔ LDL-C, Apo B, FI, HbA1c
Mensink <i>et al.</i> 2003 [30]	<i>n</i> = 60	meta-regression	13 days	1672	↓ TG, LDL-C, Apo B, TC:HDL-C ↑ HDL-C, Apo A-1 ↔ TC
Shah <i>et al.</i> 2007 [46]	<i>n</i> = 10	Random effect modell	3 weeks	400	↓ SBP, DBP *
Cao <i>et al.</i> 2009 [51]	<i>n</i> = 30	Random effect modell	2 weeks	1213	↓ TG ↑ HDL-C, Apo A 1 ↔ LDL-C
Jakobsen <i>et al.</i> 2009 [52]	<i>n</i> = 11	Random effect meta-analysis	4 years	344,696	↑ risk of CHD events ↔ risk of CHD death
Kodama <i>et al.</i> 2009 [53]	<i>n</i> = 11	Fixed effect modell	10 days	329	↓ TG ↔ FG, FI, TC, HDL-C, LDL-C
Mente <i>et al.</i> 2009 [54]	<i>n</i> = 146	Random effect meta-analysis	n.d.	101,521	↓ CHD events
Mozaffarian and Clarke 2009 [55]	<i>n</i> = 13	Multilevel regression analysis	2 weeks	554	↓ TC, TG, LDL-C, Apo B, TC:HDL-C ↑ HDL-C, Apo A-1
Skeaff <i>et al.</i> 2009 [56]	<i>n</i> = 28	Random effect meta-analysis	4 years	280,000	↔ risk of CHD death/events
Schwingshackl <i>et al.</i> 2011 [57]	<i>n</i> = 12	Random effect meta-analysis	6 months	1990	↓ FM, SBP, DBP ↔ W, WC, TC, LDL-C, HDL-C, TG, CRP
Schwingshackl <i>et al.</i> 2011 [58]	<i>n</i> = 9	Random effect meta-analysis	6 months	1547	↓ HbA1c, FG ↔ FI, HOMA-IR

↑ significant increase; ↓ significant decrease; ↔ no significant effects; * *p* = 0.05; ** MUFA vs. PUFA; MUFA/PUFA for SFA decrease
LDL-Cholesterol; n.d.: no data.

Table 6. Levels of evidence by the Scottish Intercollegiate Guidelines Network.

1++	High quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias
1+	Well conducted meta-analyses, systematic reviews, or RCTs with a low risk of bias
1–	Meta-analyses, systematic reviews, or RCTs with a high risk of bias
2++	High quality systematic reviews of case control or cohort studies
	High quality case control or cohort studies with a very low risk of confounding or bias and a high probability that the relationship is causal
2+	Well conducted case control or cohort studies with a low risk of confounding or bias and a moderate probability that the relationship is causal
2–	Case control or cohort studies with a high risk of confounding or bias and a significant risk that the relationship is not causal
3	Non-analytic studies, e.g., case reports, case series
4	Expert opinion
A	At least one meta-analysis, systematic review, or RCT rated as 1++, and directly applicable to the target population; or A body of evidence consisting principally of studies rated as 1+, directly applicable to the target population, and demonstrating overall consistency of results
B	A body of evidence including studies rated as 2++, directly applicable to the target population, and demonstrating overall consistency of results; or Extrapolated evidence from studies rated as 1++ or 1+
C	A body of evidence including studies rated as 2+, directly applicable to the target population and demonstrating overall consistency of results; or Extrapolated evidence from studies rated as 2++
D	Evidence level 3 or 4; or Extrapolated evidence from studies rated as 2+

In their meta-analysis, Clarke *et al.* (1997) [49] investigated the effects of MUFA as well as SFA and PUFA on cardiovascular risk factors in non-diabetic subjects. In addition, liquid formula diets were included, although they were analyzed separately. Dietary protocols were mostly *iso*-energetic but differed with respect to study design: they included randomized crossover, randomized or matched parallel, non-randomized Latin square and non-randomized sequential attempts. The authors concluded that substitution of carbohydrates by MUFA (5% of TEC) had no significant effect on TC and LDL-cholesterol, but managed to increase HDL-cholesterol. With respect to PUFA-rich diets, TC and LDL-cholesterol were both decreased and HDL-cholesterol was augmented in solid food experiments [49]. Clarke and Mozaffarian (2009) [55] observed that replacing hydrogenated fats with MUFA (1% of TEC) resulted in advantageous changes of several CVD risk factors like TC, LDL-cholesterol, HDL-cholesterol, TG, apoproteins A-1, B as well as B/A1, and lipoprotein (a) in 12 crossover and 1 parallel designed trials. Yu and co-workers (1995) [48] explored the results of 18 studies (again including crossover and parallel designed set-ups) enrolling a total of 804 healthy and normocholesterolemic participants. Following meta-regression, they observed that MUFA increased HDL-cholesterol and decreased TC and LDL-cholesterol. The corresponding effects of PUFA were more pronounced with respect to TC and LDL-cholesterol, but not to HDL-cholesterol [48]. In 1992, a meta-analysis of short-term RCTs investigated the effects of dietary fatty acids as an *iso*-caloric substituent for carbohydrates on CVD risk factors. HDL-cholesterol levels were significantly augmented following the MUFA-rich diet, while levels of TG and the ratio of TC to HDL-cholesterol

were significantly reduced, respectively [61]. In 2003, the authors published an updated meta-analysis including 1672 instead of 682 participants and were able to confirm their previous results. In addition, they observed a significant improvement in LDL-cholesterol, Apo A-1, and Apo B following high-MUFA regimens [30]. In a recent meta-analysis investigating the long-term (≥ 6 months) effects of high- ($>12\%$ MUFA) vs. low- ($\leq 12\%$ MUFA) MUFA diets on cardiovascular risk factors, we could show that high-MUFA diets significantly reduced systolic and diastolic blood pressure in overweight/obese subjects [57] thus confirming data previously reported by Shah *et al.* in 2007 [46]. When MUFA-rich diets were compared with PUFA-rich onsets, no effects on HDL-cholesterol and LDL-cholesterol, but a borderline increase ($p = 0.05$) in TG could be observed [47]. Hegsted *et al.* [45] analyzed metabolic studies and field trials and could not observe any impact of MUFA on TC, LDL-cholesterol, and HDL-cholesterol in their meta-regression.

5.2. Patients with Abnormal Glucose Metabolism/Diabetes Mellitus

In a recent meta-analysis of short-term RCTs (crossover and parallel study designs) with a duration between 10 days and 6 weeks enrolling 306 subjects with type 2 diabetes mellitus, a significant decrease in TG values following a MUFA-rich dietary regimen could be observed when compared with a low-fat/high carbohydrate diet [53]. This is in congruence with data presented by Garg (1998) [50] reporting reduced fasting TG in patients with type 2 diabetes mellitus subjected to a weight maintenance diet following replacement of carbohydrates by MUFA [50]. Moreover, improvements in FG and pre-prandial plasma glucose were shown, while no significant changes in fasting plasma insulin concentrations, fructosamine and HbA1c were observed. The high-MUFA protocols were accompanied with significantly lower values for TC and VLDL-cholesterol as well as increases in HDL-cholesterol, but were not correlated to changes in LDL-cholesterol. Comparison of high- ($>12\%$ MUFA) vs. low- ($\leq 12\%$ MUFA) MUFA diets on glycemic control in subjects with abnormal glucose metabolism revealed improvements in HbA1c and fasting glucose in diabetic subjects, but no differences in blood lipids were found [58,62].

With respect to short-term studies (2–12 weeks duration), comparison of low vs. moderate dietary fat content was performed in a meta-analysis by Cao *et al.* (2009) [51]. Participants with and without diabetes and a body mass index ranging from 21.1 to 30.2 kg/m² were enrolled. The mean MUFA content in a correspondingly modified diet was 23.6% of TEC and 11.4% in the low-fat versions. In the healthy collective, HDL-cholesterol was significantly increased and TG levels were significantly decreased in the moderate fat groups as compared to low-fat diets. TC and LDL-cholesterol were reduced in a similar fashion following both dietary protocols (moderate and low fat). Patients with diabetes adopting the diet with a higher MUFA content established a significant increase in HDL-cholesterol as well, accompanied by a significant reduction in TG and a non-significant reduction in TC as compared to the low fat diets. TG response was even more pronounced in participants with diabetes as compared to healthy subjects [51].

5.3. Patients with CVD

In a prospective trial investigating the effects of a Mediterranean diet, the Lyon Diet Heart Study reported a benefit of increased MUFA intake in survivors first time myocardial infarction [63]. Three

recent meta-analyses of cohort studies investigated the effects of dietary fats on CHD events and cardiovascular death. Skeaff and Miller [56] did not observe any effects of MUFA-rich diets on relative risks of CHD events and death. Moreover, no differences between of high- and low-fat intake were registered [56]. Jakobsen [52] performed a meta-analysis of cohort studies including 344,696 subjects. They postulated a positive correlation between MUFA-rich diets and risk of coronary events, but not between MUFA-rich diets and risk of coronary deaths. The authors explain that in the western diet, the MUFA supply is predominantly of animal origin resulting in a confounder that should be taken into consideration when comparing dietary fats. The usual source of MUFA/oleic acid is of vegetable origin. These results are in strong discrepancy with another recent meta-analysis of cohort studies, where Mente *et al.* [54] reported a correlation between MUFA uptake and a significant decrease in the relative risk for CHD. None of these three meta-analyses reported information regarding stroke or arrhythmic diseases, but included data for “hard” CHD endpoints like angina pectoris, sudden death, fatal and non-fatal myocardial infarction. In June 2012, the Cochrane Collaboration updated their systematic review and meta-analysis on the effects of low vs. modified fat diets on cardiovascular disease. The findings are suggestive of a small but potentially important reduction in cardiovascular risk on modification of dietary fat (but not reduction of total fat) in longer trials. However, no association between total fat content and risk of cardiovascular death and events were reported [64].

6. Conclusions

In comparison, a considerably larger number of meta-analyses explored the effects of PUFAs on maintenance or reduction of body weight as well as biomarkers of impaired glucose metabolism or CVD/CHD than there are systematic reviews and meta-analyses dealing with the corresponding impact of MUFAs. Consequently, the international recommendations for PUFA are more consistent than those for MUFA, averaging a value of 10% of TEC for healthy persons for the most part. If MUFA recommendations are given at all, they vary between 12% and 25% of TEC, equaling a remarkable range of ~30–70 g/day for a 2,500 kcal-diet. Prestigious authorities and organizations such as the National Institute of Medicine, the EFSA, the USDA and the ADA do not provide specific recommendation for MUFAs either for healthy people or for patients in need of diabetic or cardiovascular management.

In the present review, only meta-analyses were included, which indicates a high level of evidence, *i.e.*, from 2+ to 1+++ according to the Scottish Intercollegiate Guidelines Network indicate levels of evidence (Table 6). Apart from the fact that several meta-analyses and meta-regressions observed benefits of MUFA on cardiovascular risk factors, it should be noted that most meta-analyses did not report significant negative effects of a MUFA-rich diet on CVD risk factors. With respect to the favorable influences of MUFA found in studies recruiting healthy volunteers or patients with diabetes and CHD respectively, some reservations still remain. Due to various inhomogeneities, the results of different studies are far from being conclusive. Thus, MUFA were compared to carbohydrate-rich diets, low fat diets or regimens focusing on PUFA or SFA. Moreover, the term MUFA-rich diet lacks a concrete definition leading to inconsistent amounts of MUFA used in the corresponding protocols. Some of the discrepancies in the findings of different studies can be explained by their uneven and maybe incompatible durations. Long-term biomarkers of glucose metabolism such as HbA1c will be

most likely not or just slightly improved following short-term interventions of 2–6 weeks. Nevertheless, in view of the importance of dietary interventions for the prevention and therapy of cardiovascular disease, monounsaturated fatty acid may represent a valuable tool in the modification of dietary regimens. There is strong evidence that by replacing SFA and carbohydrates with MUFA, various cardiovascular risk factors will be significantly improved. The results of the different meta-analyses addressed in this review point to a beneficial effect of MUFA-rich diets on systolic and diastolic blood pressure as well as parameters of glycemic control. On the other hand, the impact of MUFA on blood lipids is still discussed controversially. While TG levels were decreased and HDL-cholesterol levels were increased following short-term interventions with higher amounts of MUFA, these findings could not be confirmed in long-term study protocols. Thus, there is no unanimous rationale for MUFAs in a therapeutic regimen. However, since no detrimental effects of MUFA-rich diets were reported in the literature to date, there is no evidence speaking against the consideration of MUFAs in dietary guidelines. Further studies dealing with long-term effects of MUFA on biomarkers of obesity, diabetes, and cardiovascular diseases as well as clinical endpoints are needed to clarify the potential benefits of MUFA-rich diets in primary and secondary prevention.

Conflict of Interest

The authors declare no conflict of interest.

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Letter to the Editor

Low-carbohydrate diets impair flow-mediated dilatation: evidence from a systematic review and meta-analysis

(First published online 5 July 2013)

With great interest, we read the meta-analysis of Bueno *et al.*⁽¹⁾ ‘Very-low-carbohydrate ketogenic diet *v.* low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials’, published ahead of print in the *British Journal of Nutrition*⁽¹⁾. In their article, the authors compared the effects of very-low-carbohydrate (VLC) diets *v.* low-fat (LF) diets on the biomarkers of obesity and their associated disorders. With respect to different outcomes between both the regimens, VLC diets turned out to be more effective in lowering body weight, TAG levels and diastolic blood pressure and in increasing HDL-cholesterol levels. However, one established risk factor of CVD, i.e. LDL-cholesterol, still turned out to be harmfully affected by the VLC regimen, most probably attributable to the larger amounts of saturated fat in the diet⁽¹⁾. In their discussion, the authors stated that future meta-analyses should investigate the impact of low carbohydrates (LC) *v.* LF on other important pathological markers, e.g. endothelial function, in order to further assess the safety of LC dietary therapies. This is reasonable, since evidence from prospective cohort studies has shown that endothelial dysfunction represents an independent risk factor for the development of many CVD including atherosclerosis⁽²⁾. We, therefore, carried out a meta-analysis to compare the effects of LC and LF regimens on flow-mediated dilatation (FMD). FMD of the brachial artery is a non-invasive measure of endothelial function, furthermore reflecting the local bioavailability of endothelium-derived vasodilators, especially NO. Inflammation of the endothelium is regarded

to play a major role in the destabilisation of atherosclerotic lesions, therefore paving the way for future CVD events⁽²⁾. A literature search was performed using the electronic databases MEDLINE (until April 2013), EMBASE (until April 2013) and the Cochrane Trial Register (until April 2013), with restrictions to randomised controlled trials, but no restriction to language using the following search terms: low fat diet AND endothelial function and low carbohydrate diet AND endothelial function (Supplementary material, available online). Studies were included in the meta-analysis if they met all of the following criteria: (1) randomised controlled design with a minimum intervention period of 3 weeks; (2) comparing a LC diet ($\leq 45\%$ carbohydrates of total energy content, TEC) with a LF diet ($\leq 30\%$ fat of TEC)⁽³⁾; (3) report of post-intervention values with standard deviations; when the results of a study were published more than once, only the most recent or most complete article was included in the analysis; (4) participants age > 18 years; (5) exclusion of subjects with CHD.

A meta-analysis was carried out in order to determine the pooled effect of the intervention in terms of weighted mean differences (WMD) between the post-intervention values of the LC group and those of the LF group. All data were analysed using the Review Manager 5.1 software, provided by the Cochrane Collaboration (<http://ims.cochrane.org/revman>). Overall, six trials with a sample size of 210 subjects were included^(4–9). Study duration ranged between 3.5 weeks and 12 months. Decreases in FMD (WMD: -1.01

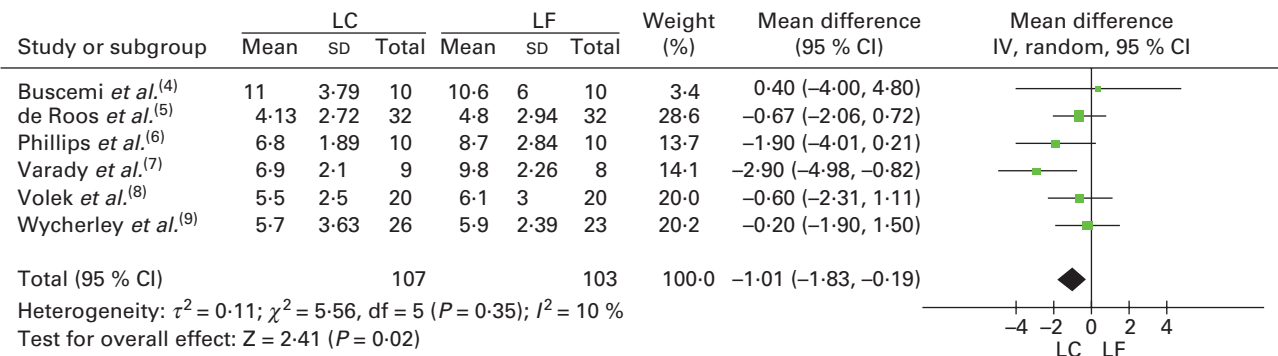


Fig. 1. Forest plot showing pooled weighted mean differences (WMD) with 95% CI for flow-mediated dilatation (%) of six randomised controlled low-carbohydrate diet studies. For each low-carbohydrate trial, ■ represents the point estimate of the intervention effects. The horizontal line joins the lower and upper limits of the 95% CI of these effects. The area of ■ reflects the relative weight of the study in the respective meta-analysis. At the bottom of the graph, ◆ represents the pooled WMD with 95% CI. (A colour version of this figure can be found online at <http://www.journals.cambridge.org/bjn>).

(95% CI -1.83 , -0.19)%, $P=0.02$) were significantly more pronounced following consumption of LC diets than following that of LF diets (Fig. 1). The I^2 test showed very low heterogeneity ($I^2 = 10\%$).

In our meta-analysis, LC dietary protocols were associated with a significant decrease in FMD when compared with their LF counterparts. A recent meta-analysis of observational studies including a sample size of 5547 subjects has observed that a 1% decrease in FMD is associated with a 13% increase in the risk of future cardiovascular events⁽²⁾. In another recent meta-analysis of cohort studies carried out by Noto *et al.*⁽¹⁰⁾, an association between LC diets and increased risk of all-cause mortality could be observed, although not for cardiovascular (CVD) mortality as well as CVD incidence⁽¹⁰⁾. It should be noted that in direct comparison with the meta-analysis carried out by Bueno *et al.*⁽¹⁾, we had to modify the inclusion criteria, since only a few dietary intervention trials had reported FMD as an outcome parameter. However, in consideration of the fact that LC was associated with a higher all-cause mortality risk, further trials are required to confirm the mechanisms of FMD impairment following LC regimens.

Supplementary material

To view the supplementary material for this article, please visit <http://dx.doi.org/10.1017/S000711451300216X>

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SYSTEMATIC REVIEW

Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: A systematic review and meta-analysis



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KEYWORDS

Cardiovascular risk factors;
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Abstract *Aim:* The aim of the present meta-analysis was to investigate the long-term effects of glycemic index-related diets in the management of obesity with a special emphasis on the potential benefits of low glycemic index/load (GI/GL) in the prevention of obesity-associated risks.

Data synthesis: Electronic searches for randomized controlled trials (RCTs) comparing low glycemic index/load versus high glycemic index/load diets were performed in MEDLINE, EMBASE and the Cochrane Library. Outcome of interest markers included anthropometric data as well as biomarkers of CVD and glycemic control. Study specific weighted mean differences were pooled using a random effect model. 14 studies were included in the primary meta-analysis. Weighted mean differences in change of C-reactive protein [WMD: -0.43 mg/dL, (95% CI -0.78 to -0.09), $p = 0.01$], and fasting insulin [WMD: -5.16 pmol/L, (95% CI -8.45 to -1.88), $p = 0.002$] were significantly more pronounced in benefit of low GI/GL diets. However decrease in fat free mass [WMD: -1.04 kg (95% CI -1.73 to -0.35), $p = 0.003$] was significantly more pronounced following low GI/GL diets as well. No significant changes were observed for blood lipids, anthropometric measures, HbA1c and fasting glucose. Sensitivity analysis was performed for RCTs excluding subjects with type 2 diabetes. Decreases in C-reactive protein and fasting insulin remained statistically significant in the low GI/GL subgroups.

Conclusions: The present systematic review provides evidence for beneficial effects of long-term interventions administering a low glycemic index/load diet with respect to fasting insulin

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and pro-inflammatory markers such as C-reactive protein which might prove to be helpful in the primary prevention of obesity-associated diseases.

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Introduction

The concept of the glycemic index (GI) was developed by Jenkins and colleagues in 1981 for management of glycemic control in patients with type 2 diabetes mellitus (T2D) [1]. The GI ranks the carbohydrate content of individual foods according to their postprandial glycemic effects expressed as a percentage of the response to an equivalent carbohydrate portion of a reference food such as 50 g of glucose. In 1997, the term glycemic load (GL) was introduced to quantify the overall glycemic effect of food with respect to its specific carbohydrate content in typically consumed quantities [2–4]. Diets with a special focus on GI/GL are usually recommended for individuals with overweight and obesity and/or patients with T2D. According to data of the World Health Organization, approximately 1.5 billion adults are considered to be either obese or overweight [5]. Besides T2D, several other metabolic disorders are known to be associated with obesity, e.g. hypertension, dyslipidemia, metabolic syndrome, and cardiovascular disease [6]. Provided that GI/GL diets represent both an effective and efficient means for weight management and glycemic control, they would represent a valuable tool in the primary and secondary prevention of these disorders. In fact, a recent meta-analysis of cohort studies showed a decreased relative risk of coronary heart disease comparing the lowest with the highest GI/GL quintile in women, but not in men [7]. Various meta-analyses of randomized controlled trials (RCTs) investigated the effects of diets with low GI/GL (LGI/LGL) as compared to high GI/GL (HGI/HGL) dietary regimens in overweight, obesity, and T2D [8–14]. In addition, high dietary GI and GL are associated with acute myocardial infarction in the Kuopio Ischemic Heart Disease Risk Factor [15]. Although a number of beneficial effects of LGI/LGL diets could be observed, most RCTs considered in the systematic reviews were short-term studies thus limiting the validity of interpretations of long-term performance of low GI protocols. Indications for a prolonged salutary effect of LGI/LGL diets were provided by a meta-analysis of prospective cohort studies reporting a significant inverse correlation between GI/GL and risk of T2D [16]. The aim of the present systematic review was to investigate the long-term (≥ 6 months) effects of LGI/LGL as a dietary means in the management of overweight and obesity with special emphasis on its potential usability in the primary prevention of obesity-associated disorders.

Methods

Literature search

Queries of literature were performed using the electronic databases MEDLINE (between 1966 and February 2013), EMBASE (between 1980 and February 2013), and the Cochrane Trial Register (until February 2013) with restrictions to randomized controlled trials, but no

restrictions to language and calendar date using the following search term: (*glycemic index*; *glycemic load*), taking variant spelling of search terms into consideration (*i.e.* *glycaemic index*; *glycaemic load*). Moreover, the reference lists from retrieved articles were checked to search for further relevant studies, and systematic reviews and meta-analysis were searched. This systematic review was planned, conducted, and reported in adherence to standards of quality for reporting meta-analyses [17].

Eligibility criteria

Studies were included in the meta-analysis if they met all of the following criteria: (1) RCTs; (2) minimum intervention period with a follow-up of 6 months; (3) comparing an LGI or LGL with an HGI or HGL dietary intervention; GI and/or GL values must have been reported; (4) assessment of the “outcome of interest” markers: body weight (BW), waist circumference (WC), fat mass (FM), fat free mass (FFM), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), triacylglycerols (TG), C-reactive protein (CRP), diastolic and systolic blood pressure (DBP, SBP), fasting glucose (FG), fasting insulin (FI) and glycosylated hemoglobin (HbA1c); (5) report of post mean or mean of two time points values with standard deviation (or basic data to calculate these parameters); (6) Body Mass Index ≥ 25 kg/m²; RCTs with type 1 diabetes mellitus (T1D) were excluded. If data of ongoing studies were published as updates, results of only the longest duration periods were included.

Risk of bias assessment

Full copies of studies were independently assessed for methodological quality by both authors using the Risk of bias assessment tool by the Cochrane Collaboration. The following sources of bias were detected: selection bias, performance/detection bias attrition bias, reporting bias and other bias [18,19] (Fig. 1).

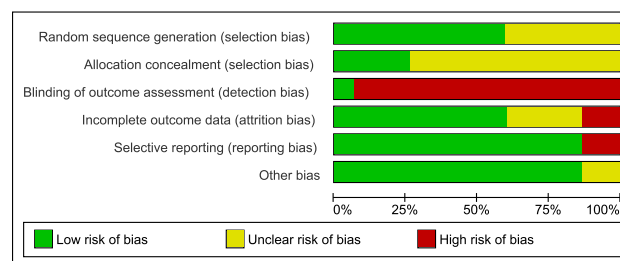


Figure 1 Risk of bias assessment tool. Across trials, information is either from trials at a low risk of bias (green), or from trials at unclear risk of bias (yellow), or from trials at high risk of bias (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Data extraction and statistical analysis

The following data were extracted from each study: the first author's last name, publication year, study duration, participant's sex and age, BMI, % diabetics, sample size, drop outs, values of GI/GL at the end of the study, outcomes and post mean values or differences in mean of two time point values with corresponding standard deviation. For each outcome measure of interest, a meta-analysis was performed in order to determine the pooled effect of the intervention in terms of weighted mean differences (WMDs) between the post-intervention (or differences in means) values of the LGI/LGL and HGI/HGL groups. Combining both the post-intervention values and difference in means in one meta-analysis is a legitimate method described by the Cochrane Collaboration [20]. All data were analyzed using the REVIEW MANAGER 5.1 software, provided by the Cochrane Collaboration (<http://ims.cochrane.org/revman>). Forest plots were generated to illustrate the study-specific effect sizes along with a 95% CI. Heterogeneity between trial results was tested with a standard χ^2 test. The I^2 parameter was used to quantify any inconsistency: $I^2 = [(Q - \text{d.f.})/Q] \times 100\%$, where Q is the χ^2 statistic and d.f. is its degrees of freedom. A value for $I^2 > 50\%$ was considered to represent substantial heterogeneity [21]. RCTs within this systematic review differ with respect to the exact GI or GL values used in the interventions. In order to detect a possible dose–response relationship between inter-study variations of LGI/LGL and HGI/HGL characteristics and changes in all outcome parameters, respectively, a random-effects meta-regression was performed. The p -values for differences in effects between the covariates were obtained using the *metareg* function of STATA 12.0 (Stata-Corp, College Station, TX, USA). Two sided p -values < 0.05 were considered to be statistically significant. Funnel plots were used to assess potential publication bias (e.g. the tendency for studies that yield statistically significant results to be more likely to be submitted and accepted for publication). To determine the presence of publication bias, we assessed the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors. Additionally, Begg's and Egger regression tests were performed [22,23]. Raw data were available from 2 RCTs [24,25]. In one trial [24], the LGI diet was approached by two different kinds of intervention: one included the LGI setting in a low fat (LF) protocol, while the other combined LGI with an high monounsaturated fat (MUFA) regime. Both types of LGI diets were included in the meta-analysis via a separate comparison of LGI/LF versus HGI/LF, LGI/MUFA versus HGI/MUFA. The same procedure was done for another RCT which compared LGI/LP (low protein) vs. HGI/LP and LGI/HP (high protein) vs. HGI/HP. This was done in order to minimize a potential confounder of LF, MUFA, LP and HP respectively, when comparing LGI and HGI diets. Data extraction was conducted independently by both authors, with disagreements resolved by consensus.

Missing data

Data processing for this review required the input of the mean and standard deviation (SD) of post-intervention values or differences in means. When SD was not available [26–28], standard errors and confidence intervals for

means were used to calculate standard deviations, according to the guidelines by the Cochrane Handbook [20].

Results

Literature search

Altogether, 15 studies extracted from 2170 articles met the inclusion criteria, and 14 of them were included in the quantitative analysis [24–37]. The paper by Raatz et al. [38] reported no appropriate follow-up data and was considered for qualitative analysis only. The detailed steps of the meta-analysis article selection process are described as a flow diagram in Fig. 2.

Study characteristics

All studies included were RCTs with a duration ranging between 24 and 68 weeks, published between 2005 and 2011 and enrolling a total of 2344 participants (65% women, 35% men). General study characteristics are summarized in (Supplementary Information Tables 1, 2).

Since type 2 diabetes mellitus (T2D) was not defined as an exclusion criteria, four studies included subjects with T2D [25–27,32]. In the LGI/LGL groups, the range for glycemic index was 30–76, while glycemic load varied between 75 and 148. The corresponding data for HGI/HGL protocols were 53–85.6 (glycemic index) and 95–280 (glycemic load).

The pooled estimate of effect size for the effects of LGI/LGL as compared to HGI/HGL on all outcomes is summarized in Table 1 (Forest plots Supplemental Figures 1–12).

Outcome parameters

Anthropometric data

Decrease in FFM was significantly more prominent following LGI/LGL dietary protocols as compared to their HGI/HGL counterparts [Weighted mean difference (WMD) -1.04 kg (95% CI -1.73 to -0.35), $p = 0.003$] ($I^2 = 0\%$). No significant changes were observed for weight [-0.62 kg (95% CI -1.28 to 0.03), $p = 0.06$] ($I^2 = 0\%$) and WC [0.06 cm (95% CI -0.83 to 0.96), $p = 0.89$] ($I^2 = 0\%$).

Blood lipids

No significant changes were observed for TC [-1.22 mg/dl (95% CI -5.62 to 3.19), $p = 0.59$] ($I^2 = 44\%$) and LDL-C [-0.29 mg/dl (95% CI -4.45 to 3.86), $p = 0.89$] ($I^2 = 54\%$), HDL-C [0.73 mg/dl (95% CI -0.23 to 1.69), $p = 0.14$] ($I^2 = 0\%$) and TG [-0.86 mg/dl (95% CI -5.65 to 3.93), $p = 0.72$] ($I^2 = 0\%$) between LGI/LGL vs. HGI/HGL diets.

Glycemic control and CRP

Decreases in CRP were more pronounced in the LGI/LGL groups as compared to their HGI/HGL counterparts [WMD: -0.43 mg/dl (95% CI -0.78 to -0.09), $p = 0.01$] ($I^2 = 54\%$) (Fig. 3). Concerning biomarkers of glycemic control, levels of FI were significantly more attenuated by LGI/LGL diets as compared to the HGI/HGL settings [WMD: -5.16 pmol/L (95% CI -8.45 to -1.88), $p = 0.002$] ($I^2 = 48\%$) (Fig. 4). None significant changes were observed for FG [WMD: 0.49 mg/dl

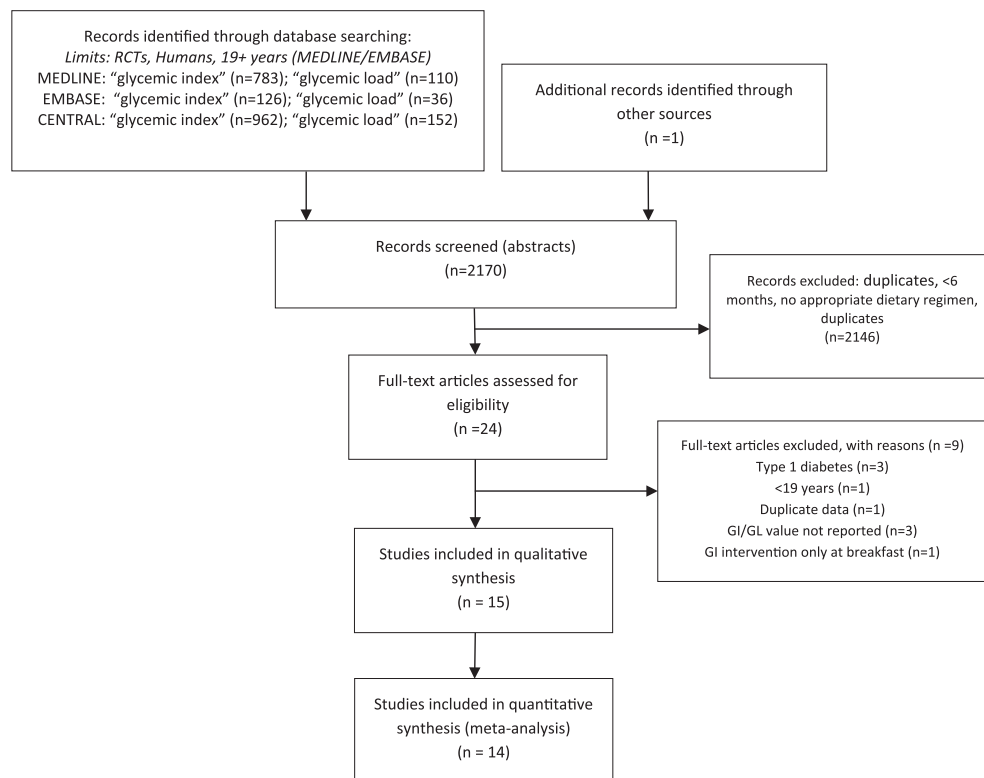


Figure 2 Flow diagram.

Table 1 Pooled estimates of effect size (95% confidence intervals) expressed as weighted mean difference for the effects of LGI/LGL vs. HGI/HGL diets on anthropometric and cardiovascular risk factors as well as glycemic control.

Outcomes	No. of studies	Sample size	WMD	95% CI	p-Values	Inconsistency I^2	Egger test
Weight (kg)	14	1770	-0.62	[-1.28, 0.03]	0.06	0%	$p = 0.289$
Weight ^a (kg)	10	1338	-0.54	[-1.22, 0.13]	0.11	0%	
WC (cm)	8	1234	0.06	[-0.83, 0.96]	0.89	0%	$p = 0.516$
WC ^a (cm)	5	1012	-0.20	[-1.22, 0.82]	0.70	0%	
TC (mg/dl)	13	1672	-1.22	[-5.62, 3.19]	0.59	44%	$p = 0.216$
TC ^a (mg/dl)	9	1254	-1.63	[-6.36, 3.11]	0.50	42%	
LDL-C (mg/dl)	14	1737	-0.29	[-4.45, 3.86]	0.89	54%	$p = 0.441$
LDL-C ^a (mg/dl)	10	1321	-0.66	[-5.21, 3.89]	0.78	55%	
HDL-C (mg/dl)	14	1734	0.73	[-0.23, 1.69]	0.14	0%	$p = 0.460$
HDL-C ^a (mg/dl)	10	1317	0.74	[-0.38, 1.86]	0.19	5%	
TG (mg/dl)	14	1735	-0.86	[-5.65, 3.93]	0.72	0%	$p = 0.152$
TG ^a (mg/dl)	10	1317	-0.87	[-5.98, 4.24]	0.74	0%	
CRP (mg/dl)	5	1204	-0.43	[-0.78, -0.09]	0.01	0%	$p = 0.386$
CRP ^a (mg/dl)	3	905	-0.41	[-0.76, -0.06]	0.02	0%	
FG (mg/dl)	10	1511	0.49	[-1.28, 2.25]	0.59	52%	$p = 0.420$
FG ^a (mg/dl)	7	1147	0.77	[-0.88, 2.43]	0.36	50%	
FI (pmol/L)	9	1132	-5.16	[-8.45, -1.88]	0.002	48%	$p = 0.008$
FI ^a (pmol/L)	7	978	-5.19	[-9.05, -1.32]	0.008	56%	
HbA1c (%)	4	421	-0.09	[-0.52, 0.33]	0.67	72%	$p = 0.958$
FFM (kg)	3	413	-1.04	[-1.73, -0.35]	0.003	0%	$p = 0.529$

^a Sensitivity analysis excluding studies enrolling patients with type 2 diabetes mellitus. CRP = high-sensitive C-reactive protein, FFM = fat free mass, FG = fasting insulin, FI = fasting insulin, HbA1c = glycosylated hemoglobin, HDL-C = high density lipoprotein-cholesterol, LDL-C = low density lipoprotein-cholesterol, TC = total cholesterol, TG = triacylglycerols, WC = waist circumference, WMD = weighted mean differences.

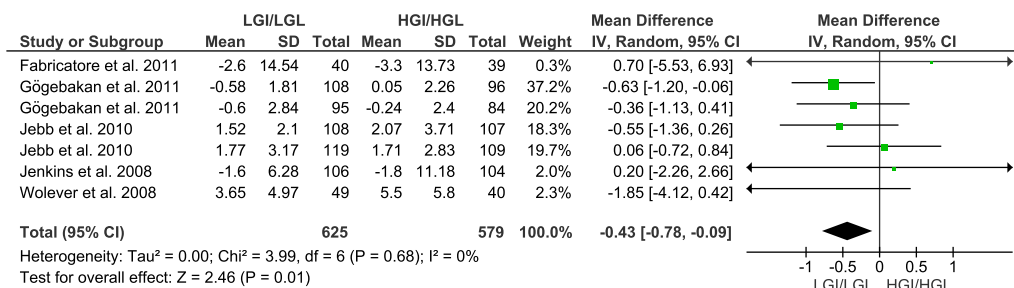


Figure 3 Forest plot showing pooled WMD with 95% CI for CRP (mg/dl) for 5 randomized controlled LGI/LGL diet studies. For each LGI/LGL study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of these effects. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled WMD with the 95% CI. Abbreviations: CRP = high-sensitive C-reactive protein, HGI/HGL = high glycemic index/load, LGL/LGI = low glycemic index/load.

(95% CI -1.28 to 2.25), $p = 0.59$ ($I^2 = 52\%$) and HbA1c [WMD: -0.09% (95% CI -0.52 to 0.33), $p = 0.67$] ($I^2 = 72\%$).

Sensitivity analysis

To investigate the effects of LGI/LGL diets on non-diabetic subjects, a sensitivity analysis was performed excluding all RCTs enrolling patients with T2D [25–27,32]. A total of 10 studies remained [24,28–31,33–37]. Changes in CRP [WMD: -0.41 mg/dl (95% CI -0.76 to -0.06), $p = 0.02$] and FI [WMD: -5.19 pmol/L (95% CI -9.05 to -1.32), $p = 0.008$] remained to be significantly more pronounced following an LGI/LGL regimen as compared to HGI/HGL diets. For FFM, no sensitivity analysis was done due to the small number of studies assessing this parameter. In addition, a subgroup analysis was performed for studies with a BMI ≥ 30 kg/m² vs. BMI < 30 kg/m² and study length ≥ 52 weeks vs. < 52 weeks. The sensitivity analysis shows overall more pronounced benefits of LGI/LGL diets (Supplemental Tables 3–6).

Publication bias

All funnel plots are provided as Supplementary Material (Supplemental Figures 13–26). The funnel plots indicate moderate asymmetry, suggesting that publication bias cannot be completely excluded as a factor of influence on

the present meta-analysis. It remains possible that small studies yielding inconclusive data have not been published. The Begg's and Egger regression tests provided no evidence of substantial publication bias, beside FI ($p = 0.008$) and FM ($p = 0.037$) (Table 1).

Heterogeneity

Moderate to substantial heterogeneity was found with respect to HbA1c ($I^2 = 72\%$), FG ($I^2 = 52\%$), LDL-C ($I^2 = 54\%$), FI ($I^2 = 48\%$), and TC ($I^2 = 44\%$) (Table 1). In spite of low heterogeneity, a random-effects meta-regression was performed to examine the associations between LGI/LGL and HGI/HGL characteristics and changes in all outcomes, respectively. No statistically significant dose-response relationship could be detected between glycemic index/glycemic load and changes in outcome parameters, but marginal dose-response relationships could be detected for TG, GL ($p = 0.07$) and GI ($p = 0.09$), respectively (Supplemental Figures 27, 28).

Discussion

The present meta-analysis investigated the effects of glycemic index/load on parameters of body composition and biomarkers of cardiovascular risk in long-term dietary

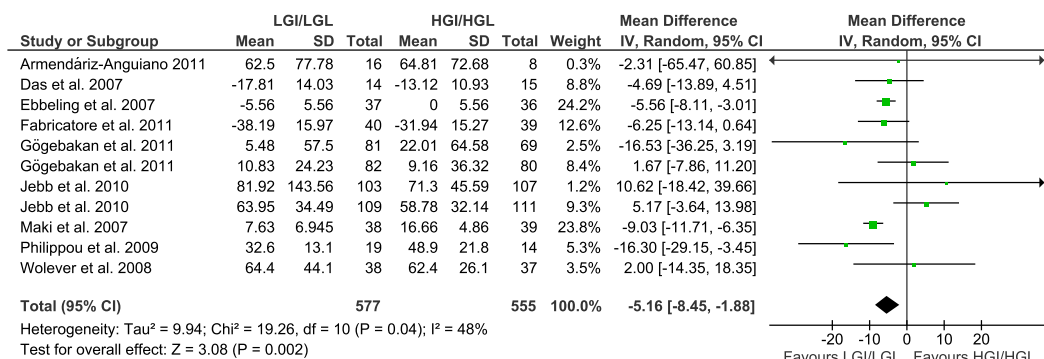


Figure 4 Forest plot showing pooled WMD with 95% CI for fasting insulin (pmol/L) for 9 randomized controlled LGI/LGL diet studies. For each LGI/LGL study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of these effects. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled WMD with the 95% CI. Abbreviations: HGI/HGL = high glycemic index/load, LGL/LGI = low glycemic index/load.

intervention studies with overweight and obese subjects. In summary, decreases in C-reactive protein, fasting insulin and fat free mass were significantly more pronounced in the LGI/LGL diet groups as compared to their HGI/HGL counterparts, while all other parameters under investigation were affected in a comparable fashion. Thus, changes in HbA1c levels did not differ between both groups, which is in contrast to data from other meta-analyses reporting a beneficial influence of LGI/LGL regimens on this predictor of CVD [12,13]. This discrepancy might be explained at least in part by the fact that these systematic reviews included data from short-term RCTs enrolling both patients with T2D and T1D. The present meta-analysis was focused on the long-term effects of GI/GL on overweight and obese individuals. Therefore, studies incorporating patients with T1D had to be excluded leaving only a small number of 4 studies assessing HbA1c. Following sensitivity analyses including non-diabetic subjects only, changes in CRP and FI remained statistically significant. Chronic, low-grade inflammation is regarded to play a major role in the pathophysiology of obesity and its associated diseases such as CVD with CRP representing an independent risk factor for the development of CVD [39,40]. The increase of CRP in obesity can be explained by macrophage infiltration into the expanded adipose tissue and subsequent increases in the production and release of macrophage-derived pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) [41,42]. The low-grade inflammatory process might be involved in insulin resistance and endothelial dysfunction thereby providing a model linking obesity and cardiovascular disease [43]. Low GI foods were shown to reduce postprandial glycemia in overweight/obese [44] as well as T2D subjects [45]. These findings might in itself provide an explanation for the link between glycemic index of foods and anti-oxidative capacity [46].

Inconsistent relationships between GI and CRP have been demonstrated in observational as well as interventional studies. Griffith et al. [47] reported no correlation between GI/GL and CRP in a prospective study in normal weight individuals, while in the subgroup with overweight and obesity, HGL was even associated with lower levels of circulating CRP. In a cross-sectional analysis of two joint observational studies performed in the Netherlands, Du et al. [48] found a positive association between GI/GL and CRP described as "borderline significant" by the authors. Likewise, correlations between GI/GL and CRP were found to be small but of considerable clinical importance in the assessment of the Women's health Initiative data [49,50]. With respect to TNF- α and IL-6, calculations of GI/GL from dietary data of the PREDIMED study revealed a significant correlation between high GI/GL and plasma levels of both cytokines at baseline. In a randomized cross-over study by Vrolix and co-workers [51], pro-inflammatory markers such as CRP, TNF- α or IL-6 were not affected by GI. In contrast, Neuhouwer et al. [52] found a significant decrease in CRP in obese volunteers subjected to an LGL dietary protocol. Moreover, the combination of aerobic exercise with either an LGI or an HGI diet resulted in significant decreases in TNF- α and IL-6 in the LGI subgroups only [53].

When regarding chronic, low-grade inflammation as a connecting link between obesity and insulin resistance, one

would expect a decrease in fasting insulin in long-term interventional trials featuring reduced levels of inflammatory markers as observed in the present meta-analysis. Systematic reviews of cohort studies suggested that HGI/HGL diets are associated with an increased risk for the development and manifestation of diabetes and CVD [16,54]. Taken together, the results of the present review suggest that LGI/LGL dietary protocol represent a valuable tool in overweight and obesity especially with regard to the prevention of obesity-associated diseases like T2D and CVD. However, decreases in FFM values turned out to more prominent following LGI/LGL protocols. Reductions in lean body mass are a common problem observed in hypo-caloric dietary interventions. The consecutive adaptations in basal metabolic rate represent an important obstacle in weight management. Therefore, the present findings might represent a serious side-effect of an LGI/LGL regimen. One should keep in mind that assessment of FFM was done in only three studies selected for this meta-analysis. Removing the study with the largest weight [28] yielded non-significant results (data not shown). It is by no means intended to create a bias in this way. Rather, the authors of the relevant study referred to technical limitations of measuring FFM via dual-energy X-ray absorptiometry. They conclude that attenuation of FFM could be attributed to fluid loss in addition to changes in lean tissue. Nonetheless, decreases in FFM remain an issue which might be resolved by a combination of dietary interventions and exercise, e.g. resistance training [55].

Funnel plots for this systematic review showed moderate asymmetry suggesting that publication bias cannot be completely excluded as a confounder of the present meta-analysis (e.g. lack of published studies with inconclusive results). A major limitation of nutritional intervention trials is the heterogeneity of various aspects and characteristics of the study protocols. In the present analysis, variations could be observed regarding type(s) of diets used, setups of LGI/LGL and HGI/HGL diets, study population (i.e. overweight, obese, T2D, abnormal glucose metabolism). To increase the power of GI/GL intake for the present analyses, data from food frequency questionnaires, 24 h dietary recalls or 3–7 day dietary protocols at the end of the study were used when available. However, variations in GI/GL still represent a potential confounder of the outcome measures. In addition, not all of the studies provided information on the quality of their respective setup (e.g. method of randomization, follow-up protocol with reasons for withdrawal) demanding a conservative interpretation of results.

In conclusion, the present meta-analysis provides evidence for a beneficial effect of long-term diets adopting a low glycemic index protocol with respect to pro-inflammatory markers such as CRP and to fasting insulin. Despite the limitations mentioned, LGI/LGL regimen might represent a useful tool in primary prevention of obesity and obesity-related complications. Therefore, this regimen should not be categorically removed from the dietary offerings for people looking for a preventive measure against these risks. However, further insights into this topic require additional long-term RCTs implementing clearly defined low and high GI/GL quantities and focusing on biomarkers of obesity-induced inflammation, e.g. adipokines.

Conflict of interest

The authors declare to have no conflict of interest.

Acknowledgments

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Appendix A. Supplementary information

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.numecd.2013.04.008>.

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RESEARCH

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Long-term effects of low-fat diets either low or high in protein on cardiovascular and metabolic risk factors: a systematic review and meta-analysis

Lukas Schwingshackl* and Georg Hoffmann

Abstract

Background: Meta-analyses of short-term studies indicate favorable effects of higher protein vs. lower protein diets on health outcomes like adiposity or cardiovascular risk factors, but their long-term effects are unknown.

Methods: Electronic databases (MEDLINE, EMBASE, Cochrane Trial Register) were searched up to August 2012 with no restriction to language or calendar date. A random effect meta-analysis was performed using the Software package by the Cochrane Collaboration Review Manager 5.1. Sensitivity analysis was performed for RCTs with a Jadad Score ≥ 3 , and excluding type 2 diabetic subjects (T2D).

Results: 15 RCTs met all objectives and were included in the present meta-analysis. No significant differences were observed for weight, waist circumference, fat mass, blood lipids (i.e. total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglycerols), C-reactive protein, diastolic and systolic blood pressure, fasting glucose and glycosylated hemoglobin. In contrast, improvements of fasting insulin was significantly more pronounced following high protein diets as compared to the low protein counterparts (weighted mean difference: $-0.71 \mu\text{U/ml}$, 95% CI -1.36 to -0.05 , $p = 0.03$). Sensitivity analysis of high quality RCTs confirmed the data of the primary analyses, while exclusion of studies with diabetic subjects resulted in an additional benefit of high-protein diets with respect to a more marked increase in HDL-cholesterol.

Conclusion: According to the present meta-analysis of long-term RCTs, high-protein diets exerted neither specific beneficial nor detrimental effects on outcome markers of obesity, cardiovascular disease or glycemic control. Thus, it seems premature to recommend high-protein diets in the management of overweight and obesity.

Keywords: High-protein, Cardiovascular risk factors, Low-fat, Glycemic control

Background

With respect to the optimal macronutrient composition in the daily diet, most international authorities recommend to increase intakes of carbohydrates at the expense of fat and protein [1,2]. However, in face of the worldwide increase in prevalence of both overweight and obesity, there is a plethora of recommendations for diets aiming at weight loss and weight management. Among them, a high-protein (HP) regimen has gained increasing interest in recent years [3]. For the general population, recommended dietary reference intakes (DRIs) for protein are $0.66 \text{ g} \cdot \text{kg body weight}^{-1} \cdot \text{d}^{-1}$ [4]. Actual consumption data for the US American population

average $1.3 \text{ g} \cdot \text{kg body weight}^{-1} \cdot \text{d}^{-1}$ in the 19–30 age group indicating a protein intake in excess of their needs [5]. The Acceptable Macronutrient Distribution Range (AMDR) for protein is given as 5–35% of daily calories depending on age [6]. A recent meta-analysis comparing HP vs. low-protein (LP) diets with a duration between 28 days and 12 months observed favorable effects of HP diets on biomarkers of obesity as well as cardiovascular risk factors such as HDL-cholesterol (HDL-C), triacylglycerols (TG), and blood pressure [7]. Several randomized controlled trials (RCTs) investigated the short-term effects of HP vs. LP diets, reporting advantages of HP protocols including a reduction in TG concentration [8–10]. A meta-regression of 87 studies concluded that low-carbohydrate, HP diets favorably affected body mass and composition independent of energy intake [11]. The benefits of HP diets might be

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explained by increased thermogenesis and satiety [12,13]. Recent data from the 26-year follow up of the Nurses' Health Study (NHS) revealed that protein sources such as red meat and high-fat dairy products were significantly associated with an elevated risk of coronary heart disease, while higher intakes of poultry, fish, and nuts correlated with a lower risk of coronary heart disease (CHD) [14]. Since there is a lack of information concerning studies with different protein contents covering a longer dietary intervention period, the aim of this meta-analysis was to compare the long-term effects of HP vs. LP regimens on biomarkers of obesity, cardiovascular complications as well as adverse effects of HP.

Methods

The review protocol has been registered in PROSPERO International Prospective Register of Systematic Reviews (crd.york.ac.uk/prospero/index.asp Identifier: CRD42012002791).

Literature search

Literature search was performed using the electronic databases MEDLINE (between 1966 and August 2012), EMBASE (between 1980 and August 2012), and the Cochrane Trial Register (until August 2012) with restrictions to randomized controlled trials, but no restrictions to language and calendar date using the following search term: (*high protein diet*). Moreover, the reference lists from retrieved articles were checked to search for further relevant studies. This systematic review was planned, conducted, and reported adhering to standards of quality for reporting meta-analyses [15]. Literature search was conducted independently by both authors, with disagreements resolved by consensus.

Eligibility criteria

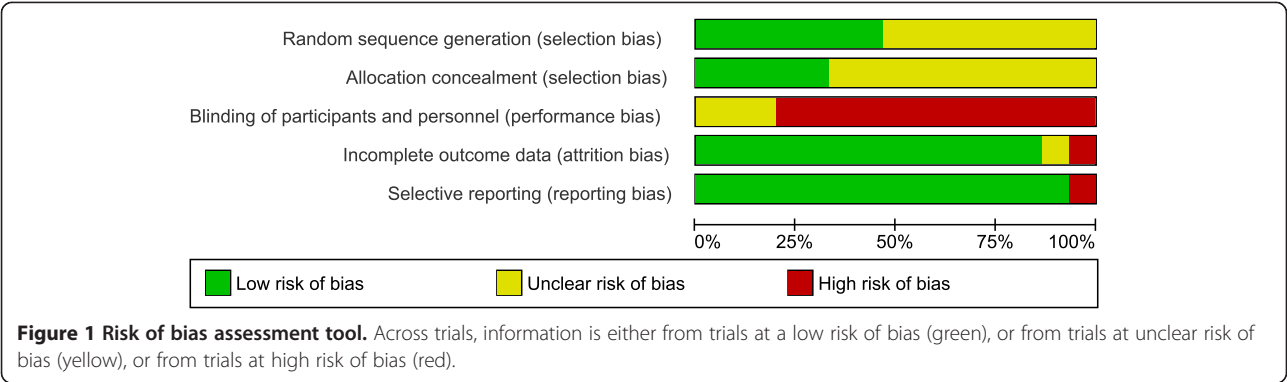
Studies were included in the meta-analysis if they met all of the following criteria: (1) randomized controlled design; (2) minimum intervention period with a follow-up of 12 months; (3) comparing a HP ($\geq 25\%$ of total energy content, TEC) with a LP dietary intervention ($\leq 20\%$ of TEC), with both protocols adopting a low fat diet ($\leq 30\%$ of TEC) [16]; (4) assessment of the outcome markers: weight, waist circumference (WC), fat mass (FM), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C, TG, diastolic and systolic blood pressure (DBP, SBP), C-reactive protein (CRP), fasting glucose (FG), fasting insulin (FI) and glycosylated hemoglobin (HbA1c); (5) report of post-intervention mean values (if not available mean of two time points were used) with standard deviation (or basic data to calculate these parameters). If data of ongoing studies were published as updates, results of only the longest duration periods were included.

Quality assessment of studies

Full copies of studies were independently assessed for methodological quality by both authors using the Jadad score [17]. This 5-point quality scale includes points for randomization (randomized = 1 point; table of random numbers or computer generated randomization = an additional 1 point), double-blinding (double-blind = 1 point; use of a placebo = additional 1 point), and follow-up (numbers and reasons for withdrawal in each group are stated = 1 point) within the report of an RCT. An additional point was accepted if the analysis was by intention-to-treat to compensate for the fact that double-blinded study protocols are elusive in dietary intervention studies. Final scores of 0–2 were considered as low quality, while final scores of ≥ 3 were regarded as representing studies of high quality. Furthermore, the trials were assessed for methodological quality using the risk of bias assessment tool by the Cochrane Collaboration [18] (Figure 1).

Data extraction and statistical analysis

The following data were extracted from each study: the first author's last name, publication year, study duration, participant's sex and age, BMI, % diabetics, sample size, outcomes, drop outs and post mean values or differences in mean of two time point values with corresponding standard deviation. Subsequently, a standardized data extraction form for this systematic review was created according to Avenell et al. [19]. For each outcome measure of interest, a meta-analysis was performed in order to determine the pooled effect of the intervention in terms of weighted mean differences (WMDs) between the post-intervention (or differences in means) values of the HP and LP groups. Combining both the post-intervention values and difference in means in one meta-analysis is a legitimate method described by the Cochrane Collaboration [20]. All data were analyzed using the REVIEW MANAGER 5.1 software, provided by the Cochrane Collaboration (<http://ims.cochrane.org/revman>). Heterogeneity between trial results was tested with a standard χ^2 test. The I^2 parameter was used to quantify any inconsistency: $I^2 = [(Q - d.f.)/Q] \times 100\%$, where Q is the χ^2 statistic and d.f. is its degrees of freedom. A value for $I^2 > 50\%$ was considered to represent substantial heterogeneity [21]. To consider heterogeneity, the random-effects model was used to estimate WMDs with 95% confidence intervals (CIs). Forest plots were generated to illustrate the study-specific effect sizes along with a 95% CI. Funnel plots were used to assess potential publication bias (e.g. the tendency for studies that yield statistically significant results to be more likely to be submitted and accepted for publication). To determine the presence of publication bias, the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors was



assessed. One study [22] imputed two types of LP diets, and these diets were combined to one group as described in the Cochrane Handbook [20]. Data extraction was conducted independently by both authors, with disagreements resolved by consensus.

Results

Literature search and characteristic of studies

A total of 15 studies extracted from 3862 articles met the inclusion criteria and were analyzed in the systematic review [22-36]. The detailed steps of the meta-analysis article selection process are given as a flow chart

in Figure 2. General study characteristics are given in Table 1. In case of more than one LP/LF group within a single study design, all LP interventions were combined as recommended by the Cochrane Collaboration [20]. Type 2 diabetes mellitus (T2D) was not defined as an exclusion criteria, and a total of three studies enrolling subjects with T2D were included in the present meta-analysis [24,32,33]. 13/15 studies reported the distribution of gender (1200 women vs. 690 men). The pooled estimate of effect size for the effects of HP as compared to LP on primary and secondary outcomes are summarized in Table 2.

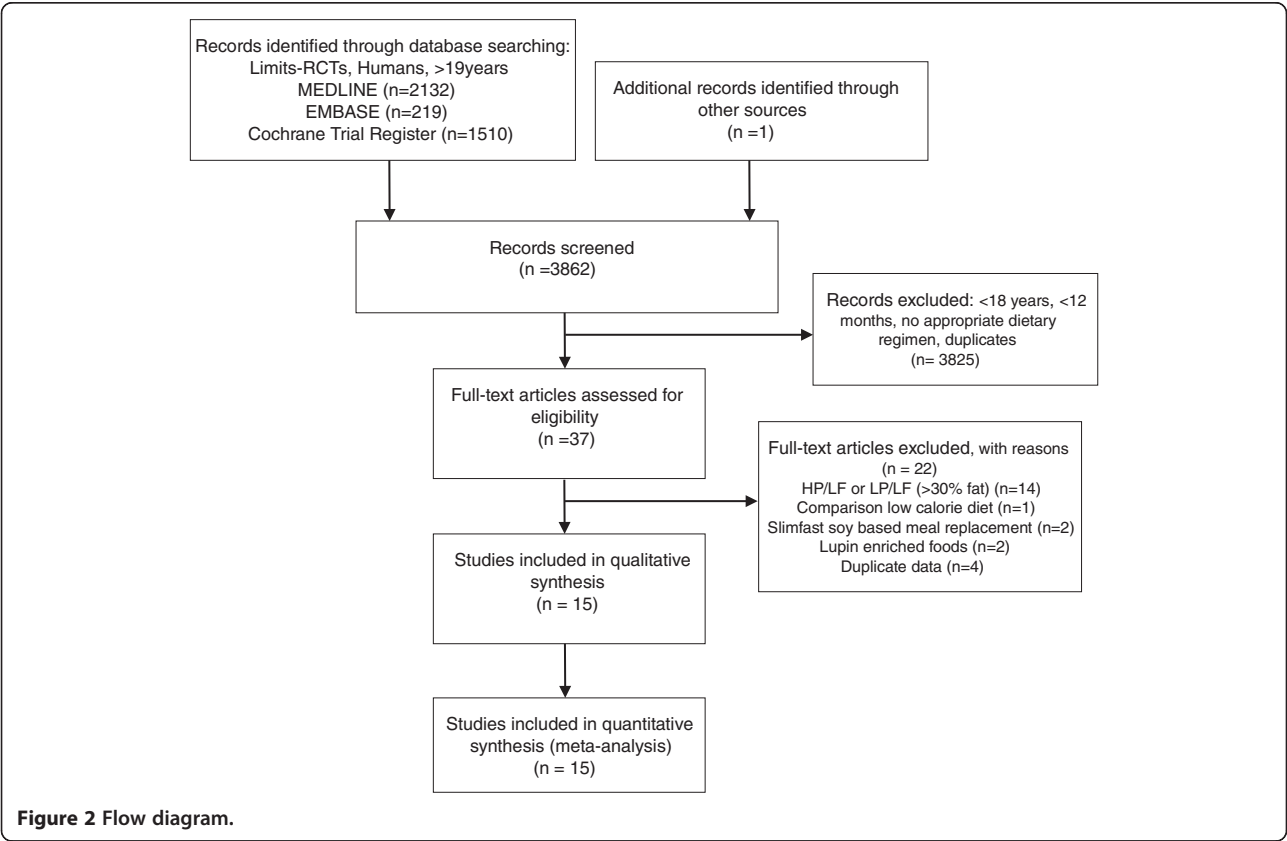


Table 1 General characteristics of randomized controlled intervention trials included in the meta-analysis

Reference	Sample size, BMI (kg/m ²), % diabetics	Age, Female (%) Male (%)	Duration, months	Dietary intervention	Dietary protocol Protein(%), Carbohydrates(%), Fat(%)	Energy restricted (kcal)	Drop Out	Study quality
Brinkworth et al. 2004 I [23]	58	50.2	16	HP/LF vs.	30%, 40%, 30%	1555 (12 weeks), energy balance (4 weeks), no restriction (follow up)	27%	2
	34	77.5%		LP/LF	15%, 55%, 30%	1555 (12 weeks), energy balance (4 weeks), no restriction (follow up)	23%	
	0%	22.5%						
Brinkworth et al. 2004 II [24]	66	>60	15	HP/LF vs.	30%, 40%, 30%	1600 (8 weeks) energy balance (4 weeks), no restriction (follow up)	39%	3
	27-40	n.d		LP/LF	15%, 55%, 30%	1600 (8 weeks) energy balance (4 weeks), no restriction (follow up)	42%	
	100%	n.d						
Clifton et al. 2007 [25]	79	49	15	HP/LF vs.	34%, 46%, 20%	1340 (12 weeks), energy balance (follow up, 52 weeks)	29%	2
	32.8	100%		LP/LF	17%, 64%, 20%	1340 (12 weeks), energy balance (follow up, 52 weeks)	38%	
	0%	0%						
Dansinger et al. 2005 [26]	80	49	12	HP/LF vs.	30%, 40%, 30%	no	35%	4
	35	48%		LP/LF	10-15%, >65%, 10%	no	50%	
	n.d	52%						
Das et al. 2007 [27]	34	35	12	HP/LF vs.	30%, 40%, 30%	1900	18%	2
	27.6	n.d		LP/LF	20%, 60%, 20%	1960	12%	
	0%	n.d						
Delbridge et al. 2009 [28]	141	44	12	HP/LF vs.	30%, 40%, 30%	no	37%	3
	39	50%		LP/LF	15%, 55%, 30%	no	41%	
	n.d	50%						
Due et al. 2004[29]	50	39.6	12	HP/LF vs.	30%, 40%, 30%	no	8%	1
	30.4	76%		LP/LF	15%, 55%, 30%	no	28%	
	0%	24%						
Gardner et al. 2007 [22]	232	40.6	12	HP/LF vs.	30%, 40%, 30%	yes	23%	4
	31.33	100%		LP/LF*	10-15%, 55-70%, 10/30%	no/yes	23%	
	0%	0%						
Keogh et al. 2007 [31]	25	48.7	12	HP/LF vs.	40%, 33%, 27%	1435	n.d	1
	32.9	68%		LP/LF	20%, 60%, 20%	1435	n.d	
	0%	32%						
Krebs et al. 2012 [32]	419	57.9	24	HP/LF vs.	30%, 40%, 30%	-500	30%	4
	36.6	60%		LP/LF	15%, 55%, 30%	-500	24%	
	100%	40%						
Larsen et al. 2011 [33]	99	59.2	12	HP/LF vs.	30%, 40%, 30%	1530 (3 months), energy balance (follow up)	19%	4
	27-40	52%		LP/LF	15%, 55%, 30%	1530 (3 months), energy balance (follow up)	20%	
	100%	48%						
Layman et al. 2008 [30]	130	45.4	12	HP/LF vs.	30%, 40%, 30%	1700 women, 1900 men	36%	2
	32.6	55%		LP/LF	15%, 55%, 30%	1700 women, 1900 men	55%	
	n.d	45%						
McAuley et al. 2006 [34]	48	n.d	12	HP/LF vs.	30%, 40%, 30%	no	7%	2
	n.d	100%		LP/LF	15%, 55%, 30%	no	25%	
	Insulin resistant	0%						
Sacks et al. 2009 [35]	406	50.5	24	HP/LF vs.	25%, 55%, 20%	-750	22%	4
	33	64%		LP/LF	15%, 65%, 20%	-750	16%	
	0%	36%						

Table 1 General characteristics of randomized controlled intervention trials included in the meta-analysis (Continued)

Wycherley et al. 2012 [36]	123	20-65	12	HP/LF vs.	35%, 40%, 25%	1700	43%	4
	27-40	0%		LP/LF	17%, 58%, 25%	1700	44%	
	0%	100%						

*two kind of LP/LF diets (very LF: 10% and LF: 30% of total energy content).
HP, high-protein; LF, low fat; LP, low-protein; n.d, no data.

Data analysis

Weight/ Waist circumference/ Fat Mass

Weighted mean differences (WMD) in change of weight [-0.39 kg (95% CI -1.43 to 0.65), $p = 0.46$], WC [-0.98 cm (95% CI -3.32 to 1.37), $p = 0.41$] and FM [-0.59 kg (95% CI -1.32 to 0.13), $p = 0.11$] were not statistically significant when comparing HP vs. LP dietary protocols.

Serum lipids

No significant changes were observed for TC [WMD: -2.51 mg/dl (95% CI -7.74 to 2.71), $p = 0.35$], LDL cholesterol [WMD: 1.58 mg/dl (95% CI -5.36 to 8.53), $p = 0.66$], HDL cholesterol [WMD: 0.90 mg/dl (95% CI -0.09 to 1.89), $p = 0.08$], and TG [WMD: -2.87 mg/dl (95% CI -11.13 to 5.38), $p = 0.49$] between HP and LP diets.

Blood Pressure/CRP

When comparing HP and LP regimen, no significant differences could be found with respect to WMDs in change of CRP [0.22 mg/dl (95% CI -0.36 to 0.79), $p = 0.46$] and blood pressure values [DBP: -0.42 mmHg (95% CI -1.37 to 0.54), $p = 0.39$; and SBP: -1.61 mmHg (95% CI -3.45 to 0.23), $p = 0.09$].

Glycemic control

Decreases in FI were significantly more explicit in subjects adhering to an HP diet as compared to those following an LP regimen [WMD: -0.71 μ IU/ml (95% CI -1.36 to -0.05), $p = 0.03$] (Figure 3). None-significant changes were observed for FG [WMD: -0.63 mg/dl (95% CI -1.93 to 0.67), $p = 0.34$], and HbA1c [WMD: 0.07% (95% CI -0.17 to 0.31), $p = 0.55$].

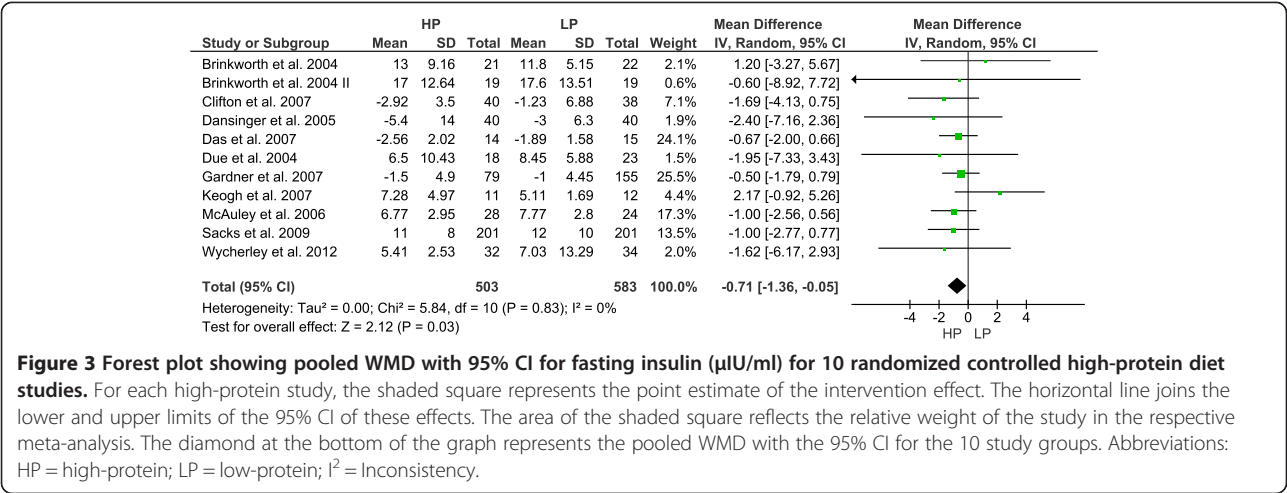
Sensitivity analysis

Articles with a Jadad quality score ≥ 3 only were included in the sensitivity analyses. A total of 8/15 studies remained for sensitivity analyses [22,24,26,28,32,33,35,36]. The results of the primary analyses could be confirmed for all the parameters that were not significantly altered in different ways in the HP and LP groups. Furthermore, changes in FI turned out to be of similar dimension as well when studies with poor Jadad scores were excluded. In an additional sensitivity analysis, studies enrolling patients with T2D [33,34,36] were discarded to account for a potential "reproducibility effect" on the pooled WMD when comparing the results of the present systematic review with a meta-analysis by Santesso et al. [7], where T2D represented an exclusion criterium. Results were not significantly different as compared to the comprehensive meta-analyses

Table 2 Pooled estimates of effect size (95% confidence intervals) expressed as weighted mean difference for the effects of HP vs. LP diets on cardiovascular and metabolic risk factors

Outcomes	No. of studies	Participants	WMD	95% CI	p-values	Inconsistency I^2
Weight (kg)	13	971	-0.39	[-1.43, 0.65]	0.46	0%
WC (cm)	8	727	-0.98	[-3.32, 1.37]	0.41	72%
FM (kg)	10	913	-0.59	[-1.32, 0.13]	0.11	0%
TC (mg/dl)	12	1251	-2.51	[-7.74, 2.71]	0.35	32%
LDL-C (mg/dl)	13	1522	1.58	[-5.36, 8.53]	0.66	79%
HDL-C (mg/dl)	14	1563	0.90	[-0.09, 1.89]	0.08	0%
TG (mg/dl)	14	1563	-2.87	[-11.13, 5.38]	0.49	21%
DBP (mmHg)	11	1402	-0.42	[-1.37, 0.54]	0.39	0%
SBP (mmHg)	11	1414	-1.61	[-3.45, 0.23]	0.09	41%
CRP (mg/dl)	4	222	0.22	[-0.36, 0.79]	0.46	0%
FG (mg/dl)	11	1357	-0.63	[-1.93, 0.67]	0.34	0%
FI (μ IU/ml)	11	1086	-0.71	[-1.36, -0.05]	0.03	0%
HbA1c (%)	3	431	0.07	[-0.17, 0.31]	0.55	0%

CI, confidence intervals; CRP, high-sensitive-C reactive protein; DBP, diastolic blood pressure; FG, fasting glucose; FI, fasting insulin; FM, fat mass; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol, TG, triacylglycerols; WC, waist circumference; WMD, weighted mean difference.



except for one parameter, i.e. the beneficial increase in HDL-C was more pronounced in the HP as compared to the LP diet [WMD: 1.50 mg/dl (95% CI 0.37 to 2.62), p = 0.009] (Figure 4).

Publication bias

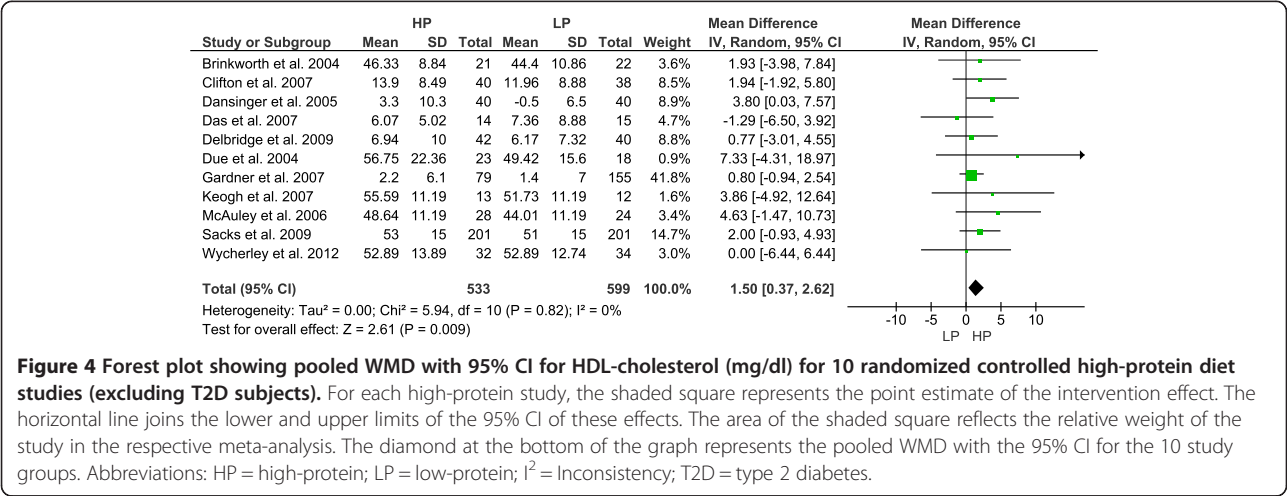
The funnel plots (with respect to effect size changes for Weight, WC, FM, TC, LDL-C, HDL-C, TG, CRP, DBP, SBP, FG, FI and HbA1c in response to HP diets) indicates little to moderate asymmetry, suggesting that publication bias cannot be completely excluded as a factor of influence on the present meta-analysis. It remains possible that small studies yielding inconclusive data have not been published.

Heterogeneity

Considerable heterogeneity was found with respect to WC (I² = 72%) and LDL-C (I² = 79%) in the primary analysis. Moderate heterogeneity was observed for TC (I² = 32%) and SBP (I² = 41%), the other outcomes showed low heterogeneity (I² = 0-21%).

Discussion

In this systematic review, HP dietary protocols were compared with LP regimens with respect to their effects on biomarkers of obesity and obesity-associated disorders such as diabetes or cardiovascular disease. Analyses were restricted on HP as well as LP diets providing ≤ 30% of TEC in the form of fat to prevent potential bias due to variations in total fat intake. Main findings suggest no advantages or disadvantages of a higher dietary protein content. None of the dietary protocols turned out to be superior to its counterpart with regard to the biomarkers under investigation. Following primary analysis, decreases in fasting insulin were significantly more pronounced in HP diets. However, this was no longer valid after inclusion of high quality trials only in the secondary analysis. The raise in HDL-C turned out to be more pronounced in the HP group compared to the LP group following sensitivity analysis excluding studies that enrolled patients with T2D. In a previous study, HP diets exerted a 12%-increase in HDL-C under closely



supervised dietary control [37]. Two meta-analyses provide evidence that higher fat intake was associated with higher levels of HDL-C when compared to low-fat diets [38,39]. With respect to the studies included in the present systematic review, the trials by Gardner et al. [22], Dansinger et al. [26], and McAuley et al. [34] reported higher intakes of total fat at the end of their 12 months protocols (dietary records) in the HP groups as compared to the respective LP counterparts. Omitting these trials to the sensitivity analysis, changes in HDL-C turned out to be similar in both HP and LP regimen (data not shown), suggesting that HDL-C response was due to dietary fat content rather than to protein consumption.

Taken together, these results are in discrepancy with a recent meta-analysis by Santesso and co-workers [7] who reported weight loss, WC, HDL-C, TG, SBP, DBP and FI to be significantly more improved following short-and long-term HP diets as compared to LP protocols. The different findings might at least in part be explained by the fact that only long-term studies with a duration ≥ 12 months were included in the present meta-analysis. In addition, both post-intervention values as well as changes in mean differences were used as suggested by the Cochrane Collaboration [20] to avoid a standardized mean differences method, whereas Santesso et al. [7] separated between primary (change from baseline values) and secondary (final values) analyses. These results indicate that HP diets do not exert favorable effects on anthropometric measures like body weight, fat mass and waist circumference. However, in a meta-regression by Krieger et al. [11] high-protein intake turned out to be a significant predictor of fat free mass retention, thereby compensating a potential side-effect of long-term energy restriction.

Dietary protein content of the high-protein diets included in this meta-analysis varied between 30-40% of TEC, which is within the age-dependent AMDR of 5-35% for all but one RCT [31]. Via analysis of the National Health and Nutrition Examinations Survey conducted between 2003 and 2004, Fulgoni [5] concluded that the actual intake of protein in US-American adults of $1.3 \text{ g} \cdot \text{kg} \text{ body weight}^{-1} \cdot \text{d}^{-1}$ exceeds the DRI values of $0.66 \text{ g} \cdot \text{kg} \text{ body weight}^{-1} \cdot \text{d}^{-1}$. He suggested that recommendations could be adapted to 25-30% of TEC, assuming benefits of higher protein intake e.g. on regulation of body weight. Regarding biomarkers such as weight, waist circumference or fat mass, the present meta-analysis does not support this concept.

Three RCTs included in this meta-analysis investigated the effects of HP regimens on biomarkers of kidney function in patients with T2D. In all trials, HP diets did not affect renal functions assessed via measurement of serum creatinine and microalbuminuria [32,33,36]. Likewise, a 2-year RCT by Friedman et al. [40] reported no harmful effects of a high-protein/low carbohydrate diet

on glomerular filtration rate, albuminuria, or fluid and electrolyte balance. With respect to prospective cohort studies, a systematic review by Mente et al. [41] indicated no significant correlations between animal protein sources, e.g. eggs, milk or meat on coronary heart disease (CHD), whereas vegetable protein sources like nuts were associated with a decreased risk. Findings from Greece, Sweden and the US noted an increased all-cause mortality following a HP/low carbohydrate diet based on animal sources in both women and men whereas a vegetable-based low-carbohydrate diet was associated with lower all-cause and cardiovascular disease mortality rates [42-44].

This systematic review did not consider unpublished data, and with respect to the moderate asymmetry of the Funnel plots, it cannot be excluded that publication bias such as lack of published studies with inconclusive results may have at least a moderate impact on the effect size estimates. An important limitation of dietary intervention trials is the heterogeneity of various aspects and characteristics of the study protocols. The literature chosen for the present meta-analysis varies regarding type(s) of diets used, definitions of HP and LP diets, study population (i.e. BMI, type 2 diabetics, abnormal glucose metabolism), intervention time, nutritional assessment as well as long-term follow-ups (between 1 and 2 yrs.). In addition, some studies were performed on hypocaloric terms, while others provided an isocaloric diet.

Not all of the studies gave details on the quality of their respective setup (e.g. method of randomization, follow-up protocol with reasons for withdrawal) yielding Jadad scores < 3 . However, following sensitivity analyses including high quality studies only (Jadad score ≥ 3), pooled estimates of effect size were similar to those obtained with the complete set of studies. Some comparisons within the present meta-analyses were done using both post-intervention values and changes in mean difference, which is considered to be a legitimate procedure as described by the Cochrane Collaboration [20], and should not be regarded as a limitation.

In summary, the present meta-analysis investigated the long-term effects of HP vs. LP both low in fat on biomarkers predicting the outcome of obesity, cardiovascular disease and glycemic control. Since biomarkers under investigation were not affected by changes in dietary protein content, unanimous recommendation of a high-protein dietary approach is not supported by evidence. With respect to the potential risk of high-protein contents, further studies are required before dietary recommendations can be changed towards a higher percentage of daily protein consumption.

Competing interests

Both Author declare that they have no competing interest.

Authors' contributions

LS and GH conducted the data analysis, interpretation of results, manuscript drafting, and finalizing manuscript. All authors read and approved the final manuscript.

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Comparison of High vs. Normal/Low Protein Diets on Renal Function in Subjects without Chronic Kidney Disease: A Systematic Review and Meta-Analysis

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Abstract

Background: It was the aim of the present systematic review and meta-analysis to investigate the effects of high protein (HP) versus normal/low protein (LP/NP) diets on parameters of renal function in subjects without chronic kidney disease.

Methods: Queries of literature were performed using the electronic databases MEDLINE, EMBASE, and the Cochrane Trial Register until 27th February 2014. Study specific weighted mean differences (MD) were pooled using a random effect model by the Cochrane software package Review Manager 5.1.

Findings: 30 studies including 2160 subjects met the objectives and were included in the meta-analyses. HP regimens resulted in a significantly more pronounced increase in glomerular filtration rate [MD: 7.18 ml/min/1.73 m², 95% CI 4.45 to 9.91, $p < 0.001$], serum urea [MD: 1.75 mmol/l, 95% CI 1.13 to 2.37, $p < 0.001$], and urinary calcium excretion [MD: 25.43 mg/24h, 95% CI 13.62 to 37.24, $p < 0.001$] when compared to the respective LP/NP protocol.

Conclusion: HP diets were associated with increased GFR, serum urea, urinary calcium excretion, and serum concentrations of uric acid. In the light of the high risk of kidney disease among obese, weight reduction programs recommending HP diets especially from animal sources should be handled with caution.

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Introduction

In face of the worldwide increase in prevalence of obesity, a large number of dietary measures aiming at weight reduction of weight management have been described. These diets differ mainly with respect to macronutrient composition, and among them, a high protein (HP) regimen has gained interest in recent years. However, there is inconsistent data regarding the potential beneficial or detrimental effects of HP diets on parameters of obesity as well as its associated risks. While HP protocols were reported to be advantageous when compared to their low/normal protein counterparts in short-term trials [1], no such benefits on outcome markers of obesity, cardiovascular disease or glycemic control could be reported in a recent meta-analysis investigating long-term interventions [2]. In 2002, the Institute of Medicine published an acceptable macronutrient distribution range (AMDR) for protein of 5–35% of daily calories (depending on age), with a special emphasis that there is insufficient data on the long-term safety of the upper limit of this range [3]. A major concern in relation to potential deleterious effects of HP diets is the increased risk of renal dysfunction [4,5]. High protein intake is regarded to be a trigger of renal hyperfiltration and may therefore

cause renal damage [6]. In animal and human studies, HP consumption has been found to accelerate chronic kidney disease (CKD), raise albuminuria and diuresis, natriuresis, and kaliuresis [7]. Epidemiological data from the Nurses' Health study showed that high intake of non-dairy animal protein may accelerate renal dysfunction in women with an already established mild renal insufficiency (glomerular filtration rate (GFR) < 80 ml/min/1.73 m²), while HP intake was not associated with a decline in regular renal function in women (initial GFR values > 80 ml/min/1.73 m²) [8]. In a long-term study in pigs, an HP diets (35% of total energy consumption, TEC) resulted in enlarged kidneys accompanied by histological damage as well as renal and glomerular volumes being 60–70% higher when compared to control animals (protein intake = 15% of TEC) [9]. Moreover, risk of kidney stone formation due to high urinary calcium excretion was increased in healthy subjects following an HP dietary protocol for 6 weeks [10]. In contrast to these findings, a 2-year trial in non-diabetic obese individuals reported that an HP diet was not associated with harmful effects on GFR, urinary albumin excretion, or fluid and electrolyte balance compared with a NP diet [11]. However, evidence indicates that obesity itself may accelerate the progression of CKD, induced by pathophysiological

mechanism such glomerular hyperfiltration/hypertrophy caused by the raised metabolic needs of the obese individual [12]. In addition, due to the National Health and Nutrition Examination Survey III (NHANES) data, approximately 30% of the US population feature characteristics of reduced kidney function ($\text{GFR} = 60\text{--}89 \text{ ml/min/1.73 m}^2$) increasing with age > 40 years [13]. Regarding the increased prevalence of overweight and obesity in this age group, HP diets might thus not represent a reasonable tool in weight management programs even for subjects without an established kidney dysfunction. Therefore, it was the aim of the present systematic review to examine the effects of HP versus LP/NP diets on parameters of renal function in adult subjects. To the best of our knowledge, this is the first meta-analysis performed to investigate the effects of HP diets on outcomes of renal function in subjects without CKD ($\text{GFR} \geq 60 \text{ ml/min/1.73 m}^2$).

Methods

Data Sources and Searches

Queries of literature were performed using the electronic databases MEDLINE (until 27th February 2014), EMBASE (until 27th February 2014), and the Cochrane Trial Register (until 27th February 2014) with restrictions to randomized controlled trials, but no restrictions to language and calendar date using the following search term: ("protein") AND ("renal" OR "kidney" OR "glomerular filtration" OR "creatinine" OR "urea" OR "albumin" OR "calcium"). Moreover, the reference lists from retrieved articles were checked to search for further relevant studies. This systematic review was planned, conducted, and reported in adherence to standards of quality for reporting meta-analyses [14]. Literature search was conducted independently by both authors, with disagreements resolved by consensus.

Study Selection

Studies were included in the meta-analysis if they met all of the following criteria: (i) randomized controlled or cross-over design; (ii) minimum intervention period of 1 week; (iii) comparing a HP dietary intervention with a NP/LP intervention (using a 5% difference in total energy intake, as defined previously by Santesso et al. 2012 [1]) that were designed for weight loss or not; (iv) age: ≥ 18 years; (v) sample size: healthy, overweight, obese, type 2 diabetes (T2D); (vi) assessment of the "outcome of interest" markers: GFR, serum creatinine, serum urea, urinary calcium excretion, urinary albumin excretion, serum uric acid, urinary pH; (vii) report of post-intervention mean values (if not available, change from baseline values were used) with standard deviation (or basic data to calculate these parameters: standard error, 95% confidence interval, p-values). If data of ongoing studies were published as updates, results of only the longest duration periods were included. Studies enrolling subjects with CKD ($\text{GFR} < 60 \text{ ml/min/1.73 m}^2$), type 1 diabetes, and macroalbuminuria were excluded. Trials were included if subjects had microalbuminuria, since data from the NHANES III indicated that 12% of the included population suffered from microalbuminuria, whereas only 1.5% had macroalbuminuria [15].

Data Extraction and Quality Assessment

The risk of bias assessment tool by the Cochrane Collaboration was applied specifying the following bias domains: selection bias (random sequence generation, allocation concealment), performance/detection bias (blinding of participants and personnel/blinding of outcome assessment), attrition bias (incomplete data outcome), and reporting bias (selective reporting) [16] (Figure S1).

The following data were extracted from each study: the first author's last name, year of publication, study length, gender distribution and age, BMI, % diabetics, sample size, protein intake (% of total energy content, TEC or $\text{g} \cdot \text{kg body weight}^{-1} \cdot \text{d}^{-1}$), protein origin, calcium intake, energy content of HP and NP/LP diets, outcomes and post mean values or differences in mean of two time point values with corresponding standard deviation.

Data Synthesis and Analysis

For each outcome measure of interest, a meta-analysis was performed in order to determine the pooled effect of the intervention in terms of weighted mean differences (MDs) between the post-intervention (or change from baseline) values of the HP and NP/LP groups. Combining both the post-intervention values and difference in means in one meta-analysis is an accepted method described by the Cochrane Collaboration [17]. All data were analyzed using the REVIEW MANAGER software provided by the Cochrane Collaboration (<http://ims.cochrane.org/revman>). The random-effects model was used to estimate MDs with 95% confidence intervals (CIs). Forest plots were generated to illustrate the study-specific effect sizes along with a 95% CI. Heterogeneity between trial results was tested with a standard χ^2 test. The I^2 parameter was used to quantify any inconsistency: $I^2 = [(Q - \text{d.f.})/Q] \times 100\%$, where Q is the χ^2 statistic and d.f. is its degrees of freedom. A value for I^2 greater than $\geq 75\%$ was considered to indicate considerable heterogeneity [18]. Funnel plots were sketched to indicate potential publication bias (e.g. the tendency for studies yielding statistically significant results to be more likely to be submitted and accepted for publication). To evaluate substantial heterogeneity, several post hoc univariate random-effects meta-regressions were performed to examine the association between age, BMI, study length, and % protein intake as independent variables, and changes in GFR, creatinine, urea, and pH (were substantial heterogeneity could be detected) as depending variables, respectively. The *p-values* for differences in effects between the covariates were obtained using the *metareg* command of Stata 12.0 (Stata-Corp, College Station, TX, USA). Two sided *p-values* < 0.05 were considered to be statistically significant. To determine the presence of publication bias, the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors were assessed.

Results

Literature Search

A total of 30 trials (32 reports) extracted from 15734 articles met the inclusion criteria and were analyzed in the systematic review (References S1). The detailed steps of the meta-analysis article selection process are given as a flow chart in Figure S2. Although in accordance with the overall inclusion criteria, one trial was excluded due to inconsistencies in the mean GFR ($\leq 60 \text{ ml/min/1.73 m}^2$ in 45% of the study population), which was considered to increase the potential for selection bias (Figure S1) [19].

Characteristics of Studies and Participants

All studies included in this systematic review were RCTs with a duration ranging between 1 week and 24 months, published between 1993 and 2013, and enrolling a total of 2160 participants. All studies compared a HP diet to a NP/LP regimen. The mean age of participants varied between 22.3 and 67 years. Protein intakes in the HP groups were mostly of animal origin except for one trial, where wheat gluten protein was used [20]. General study characteristics are given in Table 1.

Table 1. Characteristics of the included studies in the meta-analyses.

References (References S1)	Sample size	Mean age (yrs), Female, %	Duration (weeks)	Study design	Protein (g * kg body weight ⁻¹ * d ⁻¹ , % of TEC)	Protein sources in the HP group	Calcium (mg/d)	Daily energy (kcal)	Microalbuminuria	GFR- measurement
	Mean baseline BMI (kg/m ²)									
	% diabetics									
Brinkworth et al. 2004	58	50.2	68	RCT	HP: 30%	30 g skim milk powder, 60 g low-fat cheese, 200 g diet yogurt, 200 g lean meat or poultry, 250 ml low-fat milk	n.d	12-week energy restricted		According to [46,47] (ml/min)
	34	78%			NP/LP: 15%		n.d	12-week energy restricted		
	0%									
Brinkworth et al. 2010	68	51.5	52	RCT	HP: 35%	125 ml milk, 70 g cheddar cheese, 1 egg, 300 g (raw weight) beef, chicken or fish, 100 g (cooked) ham, tuna, beef, turkey, chicken, 40 g raw unsalted nuts	n.d	1433–1672		Modification in renal disease study equation was used [48] (ml/min/1.73 m ²)
	33.5	64%			NP/LP: 24%		n.d	1433–1672		
	0%									
Cao et al. 2011	16	56.9	7	Crossover	HP: 1.6 g; 20%	500 ml milk, 80 g ham, 120 g roast beef, 120 g baked chicken, 50 g steamed peas	865	Isocaloric		
	26.8	100%			NP/LP: 0.8 g; 10%		907	Isocaloric		
	0%									
Ferrara et al. 2006	15	26.4	24	RCT	HP: 1.9 g	Beef, pork, ham, poultry	n.d	n.d		
	23.5	0%			NP/LP: 1.3 g		n.d	n.d		
	0%									
Frank et al. 2009	24	24.1	1	crossover	HP: 2.4 g; 26.6%; 21.7% (animal protein)	Animal sources including milk and milk products	n.d	2743		Assessed on the basis of sinistrin clearance (ml/min)

Table 1. Cont.

References (References S1)	Sample size	Mean age (yrs)	Duration (weeks)	Study design	Protein (g * kg body weight ⁻¹ * d ⁻¹ , % of TEC)	Protein sources in the HP group	Calcium (mg/d)	Daily energy (kcal)	Microalbuminuria	GFR- measurement
Mean baseline BMI (kg/m ²)										
Female, %										
% diabetics										
Friedman et al. 2012	22.3	0%			NP/LP: 1.2 g; 13.3%; 7.4% (animal protein)		n.d	2736		
	0%									
	307	45.5	104	RCT	HP: LC diet	Unlimited protein consumption	n.d	n.d		Calculated by dividing the 24-hr urinary creatinine excretion (mg/d) by 1440 (min/ d) and then dividing again by the serum creatinine (mg/dl) x100. (ml/min)
	36.1	68%			NP/LP: 15%		n.d	1200–1500		
Gross et al. 2002	0%									
	28	57.3	4	Crossover	HP: 1.4 g	Chicken	712	n.d	x	Measured using the ⁵¹ Cr-EDTA single- injection technique (ml/min/1.73 m ²)
	26.3	25%			NP/LP: 0.66 g		732	n.d		
100%										
Jenkins et al. 2001	20	55.6	4	Crossover	HP: 27.4%; 20.1% (vegetable protein)	80 g wheat gluten protein (bread)	n.d	2764		Not described (ml/ min)
	26	25%			NP/LP: 15.6%; 8.2% (vegetable protein)		n.d	2835		
0%										
Jesudason et al. 2013	45	60.9	12	RCT	HP: 30%	90–120 g/d	n.d	1435–1674	x	Calculated from serum creatinine using the Modification of diet and renal disease study formula [49] (ml/min/1.73 m ²)
	36	22%			NP/LP: 20%		n.d	1435–1674		
100%										

Table 1. Cont.

References (References S1)	Sample size	Mean age (yrs),	Duration (weeks)	Study design	Protein (g * kg body weight ⁻¹ * d ⁻¹ , % of TEC)	Protein sources in the HP group	Calcium (mg/d)	Daily energy (kcal)	Microalbuminuria	GFR- measurement
Mean baseline BMI (kg/m ²)										
Female, %										
% diabetics										
Johnston et al. 2004	20	19–54	6	RCT	HP: 31.5%	Egg beater scramble with 28 g ham and 28 g cheese, 1 l milk, 84 open faced turkey, 28 g provolone, chicken chow mien dinner, peas and beans	1828	1700		Not described (ml/s/ m ²)
28.9										
90%										
NP/LP: 15%										
Juraschek et al. 2013	164	53.5	6	Cross-over	HP: 25%, 12.5%	Food sources used for protein replacement primarily were vegetable-based	n.d	n.d		eGFR was calculated using the CKD Epidemiology Collaboration cystatin C equation [43] (ml/min/1.73 m ²)
30.2										
45%										
NP/LP: 15%, 5.4%										
Krebs et al. 2012	419	57.8	104	RCT	HP: 30%	n.d	n.d	–500	x	
36.6										
60%										
NP/LP: 15%										
Larsen et al. 2011	99	59.4	52	RCT	HP: 30%	A combination of lean meat, chicken and fish	n.d	12-week energy restricted	x	Not described (ml/min/1.73 m ²)
27–40										
52%										
LP/NP: 15%										
12-week energy restricted										
Leidy et al. 2007	46	50	12	RCT	HP: 1.4 g; 30%	180 g cooked pork, loin, ham, or Canadian bacon	n.d	–750		Calculated from serum creatinine using the Modification of diet and renal disease study formula [49] (ml/min/1.73 m ²)
30.6										
100%										
NP/LP: 0.8 g; 18%										
–750										

Table 1. Cont.

References (References S1)	Sample size	Mean age (yrs),	Duration (weeks)	Study design	Protein (g * kg body weight ⁻¹ * d ⁻¹ , % of TEC)	Protein sources in the HP group	Calcium (mg/d)	Daily energy (kcal)	Microalbuminuria	GFR- measurement
	Mean baseline BMI (kg/m ²)	Female, %								
	% diabetics									
Li et al. 2010	100	49.3	52	RCT	HP: 2.2 g, 30%	Formula 1, Herbalife Intl., Los Angeles	n.d	–500		Not described (ml/min)
	34.5	66%			NP/LP: 1.1 g, 15%		n.d	–500		
	0%									
Liu et al. 2013	50	47.9	12	RCT	HP: LC diet	Boiled eggs 2 (ad libitum for snacks, Lactalbumin (15 g), Duck leg (220 g)	n.d	ad libitum		
	26.7	100%			NP/LP: 18%		n.d	1500		
	0%									
Luger et al. 2013	44	62.4	12	RCT	HP: 30%	Received data sheets referring to protein-rich foods: major high-protein sources included: soy-based foods, milk products, fish and poultry	n.d	1272	x	Calculated from serum creatinine using the Modification of diet and renal disease study formula [49] (ml/min/1.73 m ²)
	33.3	55%			NP/LP: 15%		n.d	1235		
	100%									
Luscombe-Marsh et al. 2005	73	20–65	16	RCT	HP: 40%	400 ml skim milk, 40 g skim milk powder, 40 g low-fat cheese, 300 g meat, poultry or fish, 20 g almonds, 200 g low-fat artificially sweetened yogurt	n.d	12-week energy restricted		(urine creatinine concentration in mmol/l x urine volume in ml/1140 min/ plasma creatinine concentration in μmol/l x 1000 ml x min) x 0.7. (ml/min)
	27–40	65%			NP/LP: 20%		n.d	12-week energy restricted		
	0%									
Noakes et al. 2005	100	49.5	12	RCT	HP: 34%	250 ml milk, 200 g low-fat yogurt, 300 g lean meat, poultry or fish	777	1342		Not described (ml/min)
	32.5	100%			NP/LP: 17%		594	134		
	0%									

Table 1. Cont.

References (References S1)	Sample size	Mean age (yrs), baseline BMI (kg/m ²)	Female, %	Duration (weeks)	Study design	Protein (g * kg body weight ⁻¹ * d ⁻¹ , % of TEC)	Protein sources in the HP group	Calcium (mg/d)	Daily energy (kcal)	Microalbuminuria	GFR- measurement
Nuttall et al. 2003;Gannon et al. 2003											
	12	n.d	n.d	5	Crossover	HP: 30%	1 l low-fat milk, 113 g beef, 255 g baked chicken, 113 g low-fat cheese, 227 g low-fat yogurt	n.d	2235	x	
	n.d	17%				NP/LP: 15%		n.d	2266		
Nuttall et al. 2006											
	8	63		5	Crossover	HP: 30%	124 g egg substitute, 56 g cheddar cheese, 226 g roasted ham, 85 g swiss cheese, 253 split pea soup, 170 g tuna, 80 g peas, 56 g dry-roasted peanuts	n.d	Isocaloric	x	
	31	0%				NP/LP: 15%		n.d	Isocaloric		
Pomerleau et al. 1993											
	20	58		3	Crossover	HP: 1.9 g; 22%; NP/LP: 0.8 g; 10%	Supplements (casein, gelatin, vegetable proteins, yeast, and soy)	883	2182	x	^{99m} Technicium-DTPA (diethylenetriamine pentaacetic acid) plasma clearance (ml/s/1.73 m ²)
	33	33%				NP/LP: 0.8 g; 10%		930	2110		
Roughead et al. 2003											
	15	60.5		8	Crossover	HP: 1.62 g; 20%; NP/LP: 0.94 g; 12%	Pork, turkey breast, beef round, ham, chicken breast	596	2296		Calculated from serum and urinary creatinine, which were measured using alkaline picric acid (ml/s)
	26.5	100%				NP/LP: 0.94 g; 12%		617	2296		
Sargrad et al. 2005											
	12	47.5		8	RCT	HP: 30%; NP/LP: 15%	Chicken, fish, eggs, low-fat milk, cheeses, nuts	567	1275	n.d	
	34.5	75%				NP/LP: 15%		521	1371		

Table 1. Cont.

References (References S1)	Sample size	Mean age (yrs), baseline BMI (kg/m ²)	Duration (weeks)	Study design	Protein (g * kg body weight ⁻¹ * d ⁻¹ , % of TEC)	Protein sources in the HP group	Calcium (mg/d)	Daily energy (kcal)	Microalbuminuria	GFR- measurement
	Mean									
	% diabetics	Female, %								
Skov et al. 1999	50	39.6	24	RCT	HP: 25%	Dairy products and meat, the latter represented by both beef, pork, poultry, lamb	n.d	ad libitum		Measured using the ⁵¹ Cr-EDTA single-injection technique (ml/min)
	30.4	76%			NP/LP: 12%		n.d	ad libitum		
	0%									
Stern et al. 2004	132	53.5	12	RCT	HP: LC diet	Unlimited: meat, fowl, fish, shellfish, eggs, 110 g hard cheese	n.d	—500		
	42.9	17%			NP/LP: 15%		n.d	no		
	41%									
Velázquez Lopez et al. 2008	41	67	4	RCT	HP: 1–1.2 g	n.d	n.d	isocaloric	x	Assessed using creatinine-clearance estimation by the Cockcroft and Gault formula [50] (ml/min)
	26.82	65%			NP/LP: 0.6–0.8 g		n.d	Isocaloric		
	100%									
Wagner et al. 2007	22	30.8–60.2	1	Crossover	HP: 2 g	Meat, dairy products, and egg white powder	n.d	Isocaloric		Calculated from serum creatinine using the Modification of diet and renal disease study formula [49] (ml/min/1.73 m ²)
	25.5	69%			NP/LP: 0.5 g		n.d	Isocaloric		
	0%									
Westman et al. 2008; Yancy et al. 2007	84	51.8	24	RCT	HP: VLC diet	Unlimited: meat, fowl, fish, shellfish, eggs, 120 g hard cheese; 60 g fresh cheese	n.d	ad libitum	x	
	38	78%			NP/LP: 15%		n.d	—500		
	100%									

Table 1. Cont.

References (References S1)	Sample size	Mean age (yrs), Female, %	Duration (weeks)	Study design	Protein (g * kg body weight ⁻¹ * d ⁻¹ , % of TEC)	Protein sources in the HP group	Calcium (mg/d)	Daily energy (kcal)	Microalbuminuria	GFR- measurement
Wycherley et al. 2012	68	50.8	52	RCT	HP: 35%	3 serves low-fat dairy, 300 g lean red meat, 100 deli-sliced meat/ canned fish	n.d	1680		According to [51] (ml/ min/1.73 m ²)
	33	0%			LP: 17%		n.d	1680		
	0%									

BMI, Body-Mass-Index; CKD, chronic kidney disease; HP, high protein; n.d, no data; NP/LP, normal/low protein; RCT, randomized controlled trial; VLC, very-low carbohydrate.
doi:10.1371/journal.pone.0097656.t001

Outcomes

The pooled estimates of effect size for the effects of HP as compared to NP/LP on outcomes of kidney function are summarized in Table 2. Changes in serum creatinine (Figure S3), urinary albumin excretion (Figure S5), uric acid (Figure S4), and urinary pH (Figure S7) were not significantly different following HP diets as compared to NP/LP diets and are given as Supplementary material.

HP diets were associated with a significantly more pronounced increase in GFR as compared to NP/LP protocols [MD: 7.18 ml/min/1.73 m² (95% CI 4.45 to 9.91), $p < 0.001$] (Figure 1). Serum urea [MD: 1.75 mmol/l (95% CI 1.13 to 2.37), $p < 0.001$] (Figure 2) and urinary calcium excretion [MD: 25.43 mg/24h (95% CI 13.62 to 37.24), $p < 0.001$] (Figure S6) were significantly more increased by HP diets in comparison to the NP/LP settings, respectively.

Sensitivity Analyses

Including RCTs investigating only subjects without T2D (18 trials) confirmed the results or the primary meta-analysis (Table S1). Furthermore, with the exception of urinary pH (only 1 trial) and urinary calcium excretion (5 trials), the main results could be confirmed when including only obese subjects (20 trials) (Table S2). Similar observations could be made for long-term trials (≥ 12 weeks, 17 trials) (Table S3). Sensitivity analysis including only T2D subjects resulted in similar observations (Table S4). Following exclusion of the trial by Jenkins et al. [20] (being the only RCT not using animal protein as a source for supplementations in HP protocols), HP diets resulted in a significantly more pronounced increase in uric acid as compared to NP/LP protocols.

Publication Bias

The funnel plots (with respect to effect size changes for markers of kidney health in response to HP diets, respectively) indicates little to moderate asymmetry, suggesting that publication bias cannot be completely excluded as a factor of influence on the present meta-analysis (Figure S8-S14). It remains possible that small studies yielding inconclusive data have not been published or failed to do so.

Heterogeneity

Considerable heterogeneity was found with respect to serum urea ($I^2 = 88\%$), urinary pH ($I^2 = 95\%$), and urinary calcium excretion ($I^2 = 90\%$) (Table 2). It was assumed that high heterogeneity might be explained by non-uniform study characteristics in the high protein groups such as variations in age, BMI, study length, and protein intake. To gain insight into these potential correlations, a random-effects meta-regression was performed to examine the associations between HP and NP/LP group parameters and changes in GFR, serum creatinine, serum urea, uric acid, urinary albumin, and urinary pH, respectively. A statistically significant dose-response relationship could be detected between protein intake and increases in serum urea ($p = 0.023$). No such correlations could be detected between the other study characteristics and parameters mentioned.

Discussion

It was the aim of the present meta-analysis to investigate the impact of HP vs. LP/NP diets on parameters of kidney function in subjects without an established CKD. The main findings suggest that subjects following an HP diet presented themselves with increased GFR, serum urea, and urinary calcium excretion, respectively. Further increases in serum concentrations of uric acid

Table 2. Pooled estimates of effect size (95% confidence intervals) expressed as weighted mean difference for the effects of HP vs. NP/LP diets on outcomes of renal function.

Outcomes	No. of Studies	Sample size	MD	95% CI	p-values	Inconsistency I ²
GFR (ml/min/1.73 m ²)	21	1599	7.18	[4.45, 9.91]	<0.001	52%
Creatinine (μmol/l)	22	1764	−1.42	[−3.50, 0.65]	0.18	57%
Urea (mmol/l)	13	910	1.75	[1.13, 2.37]	<0.001	88%
Uric acid (μmol/l)	8	295	0.18	[−0.08, 0.44]	0.17	3%
Urinary pH	7	210	−0.39	[−0.82, 0.03]	0.07	95%
Urinary Albumin/Protein (mg/24h)	11	783	0.50	[−2.83, 3.82]	0.77	63%
Urinary calcium excretion (mg/24h)	10	708	25.43	[13.62, 37.24]	<0.001	90%

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could be observed in those individuals following an HP regimen, when the trial by Jenkins et al. [20] was excluded from the analysis due to the fact, that it was the only study using vegetable protein exclusively as a supplement.

The choice of diet as a tool in weight management often includes variations in macronutrient composition differing from the regular recommendations of national as well as international authorities. Due to their proposed effects on thermogenesis and satiety, HP diets have gained increasing interest in recent years. The potential detrimental effects of HP diets on kidney function are still discussed controversially. In a long-term study in rats, feeding an HP diet (35% of TEC) resulted in a significant

reduction in body weight, however this was accompanied by 17% higher kidney weights, a 3-fold raise in proteinuria, larger glomeruli, and a 27% increase in creatinine clearance as compared to the NP (15% of TEC) feed rats, respectively [21]. Other detrimental effects of HP diets on kidney function include higher organ weight, and histologically detectable tissue damage [9]. Despite the limited transferability of results gained in animal experiments, pathophysiological side-effects of HP diets could appear in humans as well. At least in patients with established CKD, reducing protein intake decreases the occurrence of renal death by 32% when compared to higher/unrestricted protein intake [22]. Data of a meta-analysis investigating 17 cohort studies

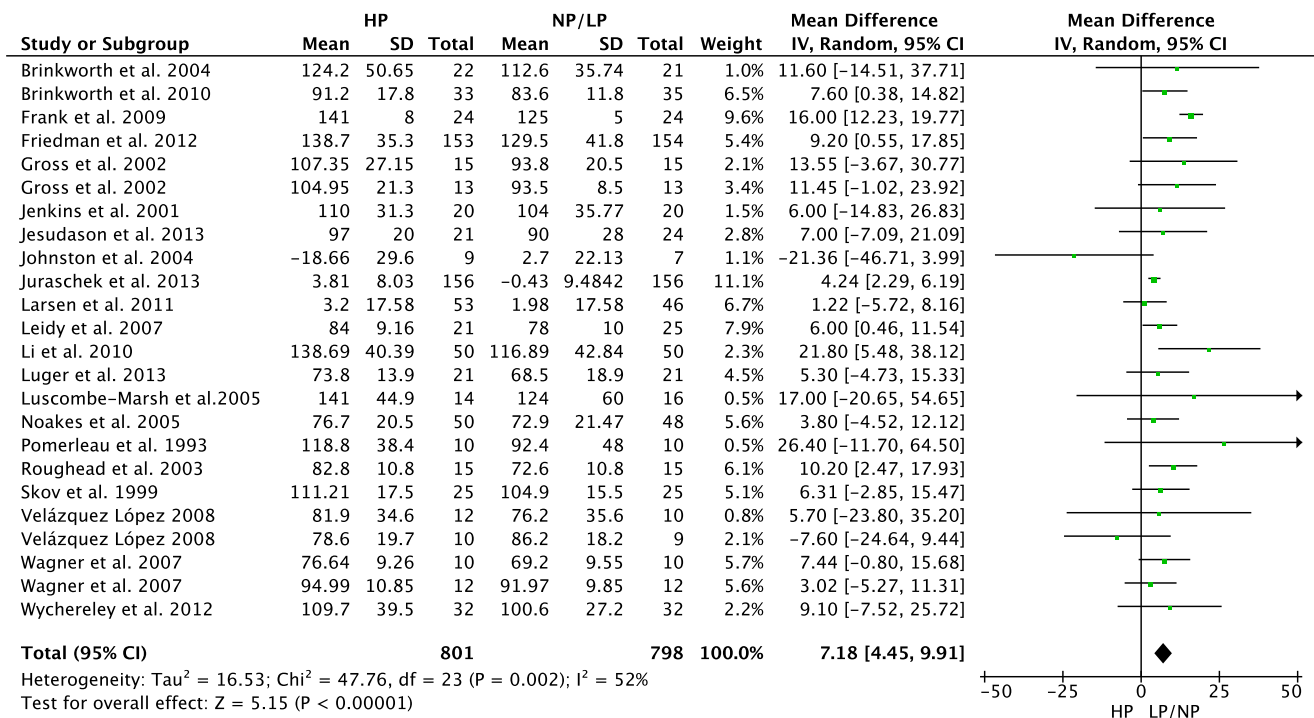


Figure 1. Forest plot showing pooled MD with 95% CI for glomerular filtration rate (ml/min/1.73 m²) of 21 randomized controlled HP diet trials. For each high protein study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of these effects. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled MD with the 95% CI. HP, high protein; NP/LP, normal protein/low protein. doi:10.1371/journal.pone.0097656.g001

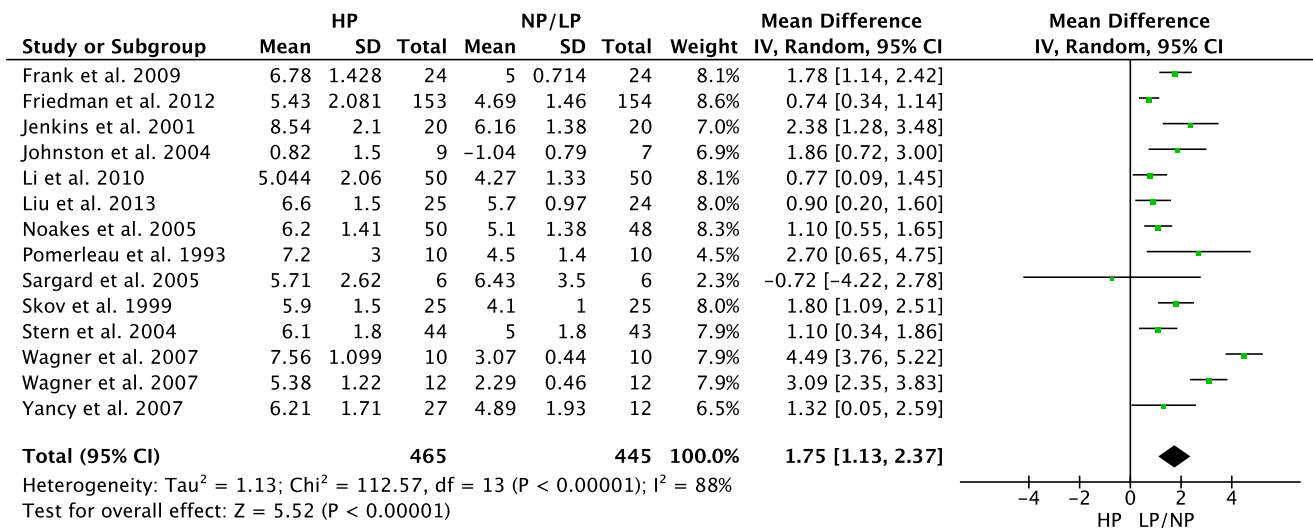


Figure 2. Forest plot showing pooled MD with 95% CI for serum urea (mmol/l) of 13 randomized controlled HP diet trails. For each high protein study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of these effects. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled MD with the 95% CI. HP, high protein; NP/LP, normal protein/low protein.
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suggest that HP/lower carbohydrate intakes were associated with increased all-cause mortality [23].

Some 30 years ago, Brenner et al. [24] expressed the hypothesis that an increase in GFR and glomerular pressure might cause renal dysfunction and raise the risk for renal injury. Although this hypothesis could neither be validated nor refuted to date, one might argue that long-term HP intakes exert harmful effects on kidney function by causing renal hyperfiltration. Concerning the mechanism mediating the increased GFR, Frank et al. [25] hypothesized that protein load induces a vasodilatory response leading to hyperemia. In a meta-analysis of 14 observational studies enrolling 105,872 participants, a $GFR > 105 \text{ ml/min/1.73 m}^2$ was associated with an increased risk of all-cause mortality [26]. However, the authors of this study stated that their findings should be interpreted conservatively. Instead of being a pathophysiological reaction, HP-induced changes in kidney function such as the increase in GFR might as well represent a physiological adaptation process [27,28]. The capacity of the kidney to increase functional level with protein intake suggest a renal function reserve [29].

The raise in serum uric acid concentrations observed in the present meta-analysis in individuals following an HP diet was most likely probably caused by the higher intake of animal source foods rich in purines. Epidemiological data suggest that protein per se does not raise serum uric acid [30]. Among others, the Health Professionals Follow-up Study observed a 41% increase in the risk of first attack of gout when comparing the highest vs. lowest meat consumption quintile [31]. In addition to gout disorders, serum uric acid has been described as a modifiable risk factor for CVD and all-cause mortality in men and women [32,33]. From these data, one may conclude that the source of protein is of higher importance than its absolute amount. A 26-year follow up of the Nurses' Health Study (NHS) revealed that protein sources such as red meat and high-fat dairy products were significantly associated with an elevated risk of CHD, while higher intakes of poultry, fish, and nuts (although rich in protein as well) were correlated with a lower risk of CHD [34]. In contrast to these findings, Bernstein et

al. [35] concluded that long-term consumption of high-protein diets may cause renal injury and accelerate the onset of CKD in persons with normal renal function independent of the fact, whether the protein food source is either predominantly animal or vegetable protein.

Reductions in urinary pH ($p = 0.07$) as observed in this meta-analysis for HP diets are regarded as an independent risk factor for nephrolithiasis [7]. In addition, HP intake raised urinary calcium excretion which is a common characteristic in patients with calcareous stones [36,37]. Impairment of calcium homeostasis might lead to a decrease in bone mineral density. However, clinical and epidemiological data do not support the concept that HP diets exert harmful effects on bone health.[1,38] Moreover, the differences observed in the present meta-analysis do not seem to be clinically relevant.

Two meta-analyses including observational studies showed that overweight, obesity and the metabolic syndrome increase the risk of kidney disease by 40 to 83% [39,40]. Considering that some two thirds the trials included in the present meta-analysis were enrolling obese subjects, it could be speculated that a high protein intake will add another detrimental factor to the increased risk of kidney dysfunction already established for this population. According to the recommendations of the American Diabetes Association, patients with T2D should not refer to HP diets as a means for weight loss due to the unknown long-term effects of protein intakes $> 20\%$ of TEC [41].

Limitations

Regarding the validity of the main outcome parameter GFR, the creatinine-based estimating equations used in the trials included in this systematic review are known to have some limitations with respect to precision as well as being affected by variations in protein intake, which might be further aggravated by the fact that the study population did not suffer from manifested chronic kidney disease. Thus, the GFR effects observed in the present meta-analysis have to be interpreted in a conservative manner, since increased creatinine values would translate into a

lower estimated GFR [42]. A cross-sectional study by Inker et al. has shown that cystatin C might represent a more useful marker for estimating GFR especially when combined with creatinine [43]. Moreover, a post hoc analysis of the “Modification of Diet and Renal Disease” study (the origin of eGFR based on serum creatinine) has shown that dietary protein reduced the change in creatinine, but did not significantly affect cystatin C changes [44].

Other limitations of the present review include the limited number of studies and the heterogeneity of the study designs. Thus, this meta-analysis does not consider unpublished data. Examination of funnel plots showed little to moderate asymmetry suggesting that publication bias cannot be completely excluded as a confounder of the present meta-analysis (e.g. lack of published studies with inconclusive results) which may have had at least a moderate impact on the effect size estimates. A major limitation of nutritional intervention trials is the heterogeneity of various aspects and characteristics of the study protocols. Therefore, it is not surprising that the RCTs and crossover studies included in the present analyses varied regarding type(s) of diets used (energy restriction, isocaloric), definitions of HP and NP/LP diets, study population (i.e. age, sex, healthy, overweight, obese, type 2 diabetics), intervention time (1–108 weeks), as well as nutritional assessment. Following sensitivity analyses excluding only trials enrolling patients with T2D, the effect of HP diets on GFR remained the same as those observed in the conclusive analyses. With respect to other potential modulating variables, sensitivity analyses and meta-regressions failed to show any correlations between the findings of the meta-analyses and age, gender, BMI, and study duration and % protein intakes (data not shown). Only few studies provided information on the quality of their respective setup (e.g. method of randomization, follow-up protocol with reasons for withdrawal, see Figure S1 for Risk of bias assessment according to the Cochrane Collaboration) demanding a conservative interpretation of results. To estimate GFR heterogeneous equations were used (see Table 2). Moreover, the included trials varied with respect to dietary assessment methods to validate adherence of participants. In an HP dietary intervention study by Friedman et al. [11], significant increases in serum creatinine clearance were found after 3 and 12 months, but were not detectable anymore following a 24 month interval, indicating that adherence to the HP diet was not present at the end of the trial. In addition, Krebs et al. [45] could not measure significant differences in renal function at any time-point (6, 12, and 24 months) when comparing an HP with a LP regimen. Assessment of protein intakes revealed that the difference between the two groups did not exceed more than 2% of TEC suggesting a very low adherence to the dietary interventions. Therefore, adherence of individuals assigned to a HP diet might change over time. Although adherence is usually good in the short term, the long-term effects of HP vs. LP/NP diets are of higher interest. Augmentations of urinary calcium excretions found in the present meta-analysis in individuals following an HP diet might be interpreted as an adherence marker of HP diets. Some of the present meta-analyses were done using both post-intervention values and changes in mean difference, however, this was considered to be an acceptable procedure as described by the Cochrane Collaboration [17]. On the other hand, this meta-analysis has several strengths as well. All analyses were conducted following a stringent protocol, e.g. participants were randomly assigned to the intervention groups in all trials. Randomized controlled trials are considered to be the gold standard for evaluating the effects of an intervention and are subject to fewer biases as compared to observational studies. With a sample size of 2160 volunteers, the present meta-analysis provides the power to

detect statistically significant mean differences as well as to assess publication bias.

In conclusion, HP diets were associated with increased GFR, serum urea, urinary calcium excretion, and serum concentrations of uric acid. Most of these changes could be interpreted as physiological adaptive mechanism induced by HP diet without any clinical relevance. However, considering of the fact that subclinical CKD is highly prevalent, and that obesity is associated with kidney disease, weight reduction programs recommending HP diets especially from animal sources should be handled with caution.

Supporting Information

Figure S1 Risk of bias assessment tool.
(EPS)

Figure S2 Flow chart.
(DOCX)

Figure S3 Forest plot showing pooled MD with 95% CI for serum creatinine.
(EPS)

Figure S4 Forest plot showing pooled MD with 95% CI for serum uric acid.
(EPS)

Figure S5 Forest plot showing pooled MD with 95% CI for urinary albumin/protein excretion.
(EPS)

Figure S6 Forest plot showing pooled MD with 95% CI for urinary calcium excretion.
(EPS)

Figure S7 Forest plot showing pooled MD with 95% CI for urinary pH.
(EPS)

Figure S8 Funnel plot: glomerular filtration rate.
(EPS)

Figure S9 Funnel plot: serum creatinine.
(EPS)

Figure S10 Funnel plot: serum urea.
(EPS)

Figure S11 Funnel plot: serum uric acid.
(EPS)

Figure S12 Funnel plot: urinary pH.
(EPS)

Figure S13 Funnel plot: urinary albumin/protein excretion.
(EPS)

Figure S14 Funnel plot: urinary calcium excretion.
(EPS)

Table S1 Sensitivity analysis for subjects without T2D.
(DOCX)

Table S2 Sensitivity analysis for obese subjects.
(DOCX)

Table S3 Sensitivity analysis for long-term studies (≥ 12 weeks).
(DOCX)

Table S4 Sensitivity analysis for T2D subjects.
(DOCX)

Checklist S1 PRISMA checklist. (DOCX)

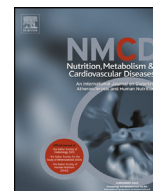
References S1 (DOCX)

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Author Contributions

Conceived and designed the experiments: LS GH. Performed the experiments: LS GH. Analyzed the data: LS GH. Contributed reagents/materials/analysis tools: LS GH. Contributed to the writing of the manuscript: LS GH.



SYSTEMATIC REVIEW

Mediterranean dietary pattern, inflammation and endothelial function: A systematic review and meta-analysis of intervention trials



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KEYWORDS

Mediterranean dietary pattern;
Inflammation;
Endothelial function;
Meta-analysis

Abstract *Background:* High adherence to a Mediterranean diet (MD) is associated with reduced all-cause and cardiovascular mortality risk. To our knowledge, there is no systematic review and meta-analysis of randomized controlled trials that has compared the effects of an MD on outcomes of endothelial function and inflammation.

Methods and results: Literature search was performed using the electronic databases MEDLINE, EMBASE, and the Cochrane Trial Register. Inclusion criteria were: randomized controlled trials, 19 + years of age, and minimum intervention period of 12 weeks. Study specific weighted mean differences (WMD) were pooled using a random effect model. Seventeen trials including 2300 subjects met the objectives. MD regimens resulted in a significantly more pronounced increase in flow mediated dilatation [WMD: 1.86%, 95% CI 0.23 to 3.48, $p = 0.02$; $I^2 = 43\%$], and adiponectin [WMD: 1.69 $\mu\text{g/ml}$, 95% CI 0.27 to 3.11, $p = 0.02$; $I^2 = 78\%$], while high-sensitive C reactive protein [WMD: -0.98 mg/l , 95% CI -1.48 to -0.49 , $p < 0.0001$; $I^2 = 91\%$], interleukin-6 [WMD: -0.42 pg/ml , 95% CI -0.73 to -0.11 , $p = 0.008$; $I^2 = 81\%$], and intracellular adhesion molecule-1 [WMD: -23.73 ng/ml , 95% CI -41.24 to -6.22 , $p = 0.008$; $I^2 = 34\%$] turned out to be significantly more decreased.

Conclusion: The results of the present meta-analysis provide evidence that an MD decreases inflammation and improves endothelial function.

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Introduction

The traditional Mediterranean diet (MD) first postulated by Ancel Keys in the 1960s [1] is defined by a high intake of virgin olive oil, vegetables, fruits, plant proteins, whole grains, fish, low-fat dairy, moderate alcohol intake, and low red meat consumption [2,3]. The Prevención con Dieta Mediterránea, a large intervention trial including 7447

subjects, showed that an MD rich in olive oil or nuts reduced the risk of cardiovascular disease (CVD) by 30% when compared to the low fat counterparts [4]. Systematic reviews investigated the effects of an MD on cardiovascular risk factors in overweight, obese and type 2 diabetic (T2D) subjects reporting improvements of these outcome parameters [5–9].

Low-grade, chronic inflammation is essential in the development of atherosclerosis, the main cause of coronary heart disease. Inflammation appears to play a key role from the initial phase to the final lesions of this disease [10] and is characterized by the infiltration of macrophages, deposition of lipids, and thickening of the vascular

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wall in response to chemo-attractant cytokines [11]. Increased expression and activation of C-reactive protein (CRP), or pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) are associated with the development of atherosclerosis. A meta-analysis reported a significant association between elevated levels of IL-6, CRP and T2D risk, furthermore supporting the hypothesis that chronic inflammation is a predictor of T2D development [12]. Evidence from prospective cohort studies showed that endothelial dysfunction represents an independent risk factor for the development of many CVD [13]. Inflammation of the endothelium is regarded to play a major role in the destabilization of atherosclerotic lesions paving the way for future CVD events [13]. There is a causal link between inflammation, endothelial dysfunction and atherosclerosis. A meta-analysis reported a reduction of CRP for low- as compared to high glycemic index/load diets [14]. Furthermore, low carbohydrate diets were reported to impair flow mediated dilatation when compared to the corresponding effects of low fat diets [15]. From these observations, one may conclude that the decreased CVD mortality following MD can be explained by beneficial counter-regulatory effects of this regimen on inflammation and endothelial dysfunction. A review by the Cochrane Group concluded that the MD pattern reduces some cardiovascular risk factors in the primary prevention of CVD [16]. However, to our knowledge, there is no systematic review and meta-analysis of randomized controlled trials (RCTs) comparing the effects of an MD on outcomes of endothelial function and inflammation. Thus, it was the aim of the present study to summarize the available data regarding the impact of MD on these parameters.

Methods

Search strategy

Queries of literature were performed using the electronic databases MEDLINE, EMBASE, and the Cochrane Library until February 22nd, 2014 with restrictions to RCTs, but not to language and calendar date using the following search terms: (“Mediterranean diet” or “diet” or “Mediterranean” or “score” or “pattern” or “adherence”) in combination with (“inflammation” or “endothelial” or “vascular”). This systematic review was planned, conducted, and reported in adherence to standards of quality for reporting meta-analyses [17]. Literature search was conducted independently by both authors, with disagreements resolved by consensus.

Selection criteria

Studies were included in the meta-analysis if they met all of the following criteria: (i) randomized controlled or cross-over design; (ii) minimum intervention period of 12 weeks; (iii) MD dietary intervention or provision of dietary factors relevant to the MD with at least two components from the following list required:

- High monounsaturated/saturated fat ratio (use of olive oil as main cooking ingredient),
- Low to moderate red wine consumption,
- High consumption of legumes,
- High consumption of grains and cereals,
- High consumption of fruits and vegetables,
- Low consumption of meat and meat products and increased consumption of fish,
- Moderate consumption of milk and dairy products;

(iv) comparison group was no intervention or minimal intervention ((ii) – (iv) according to Rees et al., 2013 [16]); (iv) participants ≥ 19 years of age; (v) sample size: no exclusion criteria (sensitivity analyses were performed according to study populations); (vi) assessment of the “outcome of interest” markers descriptive of chronic inflammation (CRP, IL-6, adiponectin, AD) and endothelial function (intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-Selectin, FMD); (vii) report of change from baseline values (if not available, post-intervention mean values were used instead) with standard deviation (or basic data to calculate these parameters: standard errors, 95% confidence interval, *p*-values). If data of ongoing studies were published as updates, results of only the longest duration periods were included.

Data extraction and quality assessment

The risk of bias assessment tool by the Cochrane Collaboration was applied specifying the following bias domains: selection, performance/detection, attrition, and reporting bias [18] (Supplemental Fig. S1). The following data were extracted from each study: the first author’s last name, year of publication, study length, gender distribution and age, BMI, % diabetics, sample size, study design, description of MD intervention and comparison group, energy content of diets, outcomes and post mean values or differences in mean of two time point values with corresponding standard deviation. Data extraction was conducted by one author (L.S.), whereas quality assessment was independently performed by both authors (L.S. and G.H.), with disagreements resolved by consensus.

Data synthesis and analysis

For each outcome parameter, a meta-analysis was performed in order to determine the pooled effect of the intervention in terms of weighted mean differences (WMDs) between the change from baseline or post-intervention mean values of the MD and control groups. Combining both data in one meta-analysis is an accepted method described by the Cochrane Collaboration [19]. All data were analyzed using the REVIEW MANAGER 5.1 software, provided by the Cochrane Collaboration (<http://ims.cochrane.org/revman>). The random-effects model was used to estimate WMDs with 95% confidence intervals (CIs). Forest plots were generated to illustrate the study-specific effect sizes along with a 95% CI. Data were

pooled if outcomes were reported by at least two studies. Heterogeneity between trial results was tested with a standard χ^2 test. The I^2 parameter was used to quantify any inconsistency: $I^2 = [(Q - \text{d.f.})/Q] \times 100\%$, where Q is the χ^2 statistic and d.f. is its degrees of freedom. A value for $I^2 \geq 75\%$ was considered to indicate considerable heterogeneity [20]. Moreover, to investigate possible sources of heterogeneity across studies, we performed a meta-regression analysis to investigate the effects of various characteristics (age, BMI, study length, sample size) of trials on the study estimates of WMDs (if $n \geq$ five trials), using the *metareg* command (STATA 12.0, Stata-Corp, College Station, TX, USA).

Funnel plots were sketched to indicate potential publication bias. To determine the presence of publication bias, the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors was assessed. In addition, Begg's and Egger regression tests were performed to test for potential publication bias [21,22]. When it was not possible to retrieve adequate data, imputations were performed [19].

Results

Literature search

A total of 17 trials (20 reports) met the inclusion criteria and were analyzed in the systematic review [23–42]. The detailed steps of the meta-analysis article selection process are depicted as a flow chart (Supplemental Fig. S2).

Characteristics of studies and participants

All studies were RCTs with a duration ranging between 12 weeks and 48 months, published between 2003 and 2013, and enrolling a total of 2300 subjects. The mean age of participants varied between 25 and 77 years. Detailed study characteristics are given in Table 1.

Outcomes

The pooled estimates of effect size for the effects of MD as compared to control interventions on outcomes of inflammation and endothelial function are summarized in Table 2. Changes in E-Selectin were not significantly different following MD as compared to control interventions.

Inflammatory markers

MD were associated with a significantly more pronounced reduction in CRP [WMD: -0.98 mg/l, 95% CI -1.48 to -0.49 , $p < 0.0001$; $I^2 = 91\%$] and IL-6 [WMD: -0.42 pg/ml, 95% CI -0.73 to -0.11 , $p = 0.008$; $I^2 = 81\%$] as compared to control intervention protocols, respectively. Plasma levels of AD were significantly more increased in subjects adhering to an MD when compared to control diets [WMD: 1.69 μ g/ml, 95% CI 0.27 to 3.11 , $p = 0.02$; $I^2 = 78\%$] (see Supplemental Figs. S3, S4, and S5).

Endothelial function

With respect to endothelial function, FMD values [WMD: 1.86% , 95% CI 0.23 to 3.48 , $p = 0.02$; $I^2 = 43\%$] were significantly more augmented while ICAM-1 concentrations [WMD: -23.73 ng/ml, 95% CI -41.24 to -6.22 , $p = 0.008$; $I^2 = 34\%$] were significantly more decreased in subjects adhering to an MD following comparison to control interventions (see Supplemental Figs. S6, and S7).

Sensitivity analyses/subgroup analyses/meta-regression

Sensitivity analysis excluding secondary prevention trials [35,39], moderate chronic renal failure [41], symptomatic asthma [37], and rheumatoid arthritis [38] confirmed the results of the primary analysis (Supplemental Table 1). Subgroup analyses (performed only for CRP and IL-6) for change scores vs. post-intervention values, study length (short-term trials, <24 weeks vs. long-term, ≥ 24 weeks), low-bias risk vs. high-risk bias, sample size (≥ 100 vs. < 100), and obese vs. non-obese were performed (Supplemental Figs. S8–S17). Pooling change from baseline values, long-term trials, larger sample size, and non-obese subjects resulted in significant reductions of CRP and IL-6 when compared with their counterparts. Reductions for CRP could be observed only for low-risk of bias trials, whereas reductions for IL-6 were statistically significant only in the high risk of bias trials. The subgroup results for IL-6 of change scores (five trials) vs. post-intervention values (one trial), and low (four trials) vs. high-risk (two trials) were based on comparison among few studies. Univariate meta-regression analyses were performed (only CRP and IL-6 provided sufficient data) by grouping studies according to sample size, BMI, age of participants, and length of follow-up (Supplemental Table 2). There was a trend for a borderline significant association between decrease of IL-6 and study length ($p = 0.054$) (Supplemental Fig. S18).

Publication bias

Funnel plots were generated for outcome measures provided by at least 10 different trials (only CRP). The plot indicates little to moderate asymmetry, suggesting that publication bias cannot be completely excluded as a factor of influence on the present meta-analysis (see Supplemental Fig. S19). It remains possible that small studies yielding inconclusive data have not been published or failed to do so. However, the Begg's and Egger regression tests provided no evidence of substantial publication bias for CRP and IL-6 (Table 2).

Discussion

The present systematic review and meta-analysis showed that a Mediterranean dietary pattern significantly improved markers of inflammation such as CRP, IL-6, and AD as well as parameters of endothelial function measured as FMD and ICAM-1. Regarding the validity of these outcome parameters, consistent evidence indicates that

Table 1 General study characteristics.

Reference	Sample size, mean baseline BMI (kg/m ²) % diabetics	Mean age (yrs), female (%)	Duration (weeks)	Study design	Health status	MD intervention	Comparison group
Athyros et al., 2011 [23]	100 27.6 0%	54.6 50%	16	RCT	Hypercholesterolemic (TC: 200–250 mg/dl)	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ olive oil; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ dairy products	Placebo
Blumenthal et al., 2010 [24]	95 32.9 0%	51.8 66%	16	RCT	Participants were overweight or obese with untreated hypertension (SBP 130–159 mmHg, DBP 85–99 mmHg based on 4 screening visits)	Low in saturated fat, cholesterol and total fat; focused on fruits, vegetables, and fat-free or low-fat dairy products; was rich in whole grains, fish, poultry, beans, seeds and nuts; contained fewer sweets, added sugars and sugary beverages, and red meats than the typical American diet. Participants in the intervention group received counseling on the DASH diet and provided feedback on their adherence in weekly group sessions. The goal of the sessions was to assist participants in learning how to buy and prepare appropriate foods, enhance their motivation to choose to eat these foods and to overcome any obstacles. A dietitian made the recommendations and small group sessions were held weekly (30–45 min each) at the research centre. Immediately after randomization and before the counseling sessions participants entered a 2-week controlled isocaloric feeding period to improve compliance with the DASH diet	The comparison group were asked to maintain their usual dietary and exercise habits. Follow-up was at 4 months after the intervention period
de Mello et al., 2011 [25]	70 31	59 51%	12	RCT	IFG (5.6–6.9 mmol/L) or IGT (7.8–11 mmol/L during an OGTT-75 g glucose); and at least two of the following. 1) BMI: 26–39 kg/m ² 2) waist circumference 102 cm (men) or 88 cm (women)); 3) low levels of serum HDL-cholesterol (40 mg/dL (men) or 50 mg/dL (women)); 4) hypertriglyceridaemia (triglycerides level of ≥150 mg/dL); (4) elevated blood pressure (≥130/85 mmHg)	Replace their usual cereal products by products with at least 50% of their composition from a whole grain source. The breads recommended, which contributed 20%–25% of total energy intake, were: a selection of commercial rye breads, sourdough whole-meal wheat bread, and also endosperm rye bread. The recommended intake of whole-meal pasta was at least 35 g (uncooked) per week. In addition, one daily portion of another cereal product habitually consumed by the participants was allowed. Participants were instructed to eat fatty fish (100–150 g fish per meal) three times per week, and to use vegetable oil and vegetable oil-based products in fish preparation. Otherwise, we did not seek to change the quality of the dietary fat, except for an increase in fatty fish consumption. Bilberries were advised to be eaten as three portions per day (equivalent of 300 g fresh bilberries per day). Participants were allowed to retain one of their habitual berry portions (e.g. raspberries) three to four times per week	In the control group, participants were asked to avoid whole grain cereals. They were also instructed to replace the breads they usually consumed with refined wheat breads, and other cereal products, e.g. porridge or pasta, with low-fiber products. The intake of bilberries was not allowed and consumption of fatty fish was allowed once a week only
Djuric et al., 2009 [26]	69 Non-obese 0%	25–65 100%	24	RCT	Healthy, non-smoker, and in the normal to overweight range (BMI: 18.5–30 kg/m ²)	Greek Mediterranean exchange list diet with exchange goals determined by dietitians at baseline and focused on increasing fruit and vegetable intake and variety and increasing MUFA intake while maintaining the baseline energy intake and total fat intake. The fruit and vegetable goal was 7–9 servings/day depending on baseline calorie intake and maintaining baseline energy intake was achieved by substituting fruit and vegetables for other carbohydrates. Variety was achieved using exchange lists. The fat intake goal was PUFA:SFA:MUFA ratio of 1:2:5. This was achieved by reducing usual fat intakes by half using low-fat food and then adding in olive oil or other high MUFA to the diet to keep energy and total fat intake at baseline levels. Participants were given 3 L of extra-virgin olive oil at baseline and at 3 months. 7-day food records were taken at baseline, 3 months and 6 months. Counseling by the dietitians occurred weekly by telephone for the first 3 months and twice weekly thereafter. Face-to-face counseling occurred at baseline and 3 months	The comparison group followed their usual diets. They did not receive counseling, but were given the National Cancer Institute's Action guide to healthy eating and written materials on nutritional deficiencies if below 67% RDA. Follow-up was at 6 months after the end of the intervention period

Esposito et al., 2003 [28]	120 34.85 0%	34.6 100%	104	RCT	Obese were sedentary (<1 h/wk of physical activity)	Program involved education on reducing dietary calories, personal goal setting, and self-monitoring through a series of monthly small-group sessions. Behavioral and psychological counseling was also offered. The mean caloric intake goal was set at 1300 kcal/d for the first year and 1500 kcal/d for the second year. The recommended composition of the dietary regimen was 50%–60% carbohydrates, 15%–20% proteins, less than 30% total fat, less than 10% SFA, 10%–15% MUFA, 5%–8% PUFA, and 18 g of fiber per 1000 kcal. Dietary advice was tailored to each woman on the basis of 3-day food records. These women also received individual guidance on increasing physical activity, mainly by walking, but also with swimming or aerobic ball games. Women were enrolled in the program for 24 months and had monthly sessions with the nutritionist and exercise trainer for the first year and bimonthly sessions for the second year. Compliance was assessed by attendance at the meetings and completion of the diet diaries	General oral and written information about healthy food choices and exercise at baseline and at subsequent monthly visits, but no specific individualized programs were offered to them
Esposito et al., 2004 [27]	180 28 0%	43.85 45%	104	RCT	Metabolic syndrome Inclusion criteria: 3 of the following: (1) abdominal adiposity (defined as waist circumference 102 cm (men) or 88 cm (women)); (2) low levels of serum HDL-cholesterol (40 mg/dL (men) or 50 mg/dL (women)); (3) hypertriglyceridaemia (triglycerides level of ≥ 150 mg/dL); (4) elevated blood pressure ($\geq 130/85$ mmHg); and (5) impaired glucose homeostasis (fasting plasma glucose concentration ≥ 110 mg/dL)	Through a series of monthly small-group sessions, participants received education in reducing dietary calories (if needed), personal goal-setting and self-monitoring using food diaries. Behavioral and psychological counseling was also offered. Dietary advice was tailored to each participant on the basis of 3-day food records. The recommended composition of the dietary regimen was carbohydrates, 50–60%; proteins, 15–20%; total fat, < 30%; SFA, < 10%; and cholesterol consumption, < 300 mg/day. Participants were advised to consume at least 250–300 g of fruits, 125–150 g of vegetables, 25–50 g of walnuts, 400 g of whole grains (legumes, rice, maize and wheat) daily and to increase their consumption of olive oil. Participants were in the program for 24 months and had monthly sessions with the nutritionist for the first year and twice monthly sessions for the second year. Compliance with the program was assessed by attendance at the meetings and completion of diet diaries	Given general oral and written information about healthy food choices at baseline and at subsequent visits. The general recommendation for macro-nutrient composition of the diet was similar to that for the intervention group (carbohydrates, 50–60%; proteins, 15–20% and total fat, 30%). Participants had bimonthly sessions with study personnel
Estruch et al., 2006 [29] Corella et al., 2009 [30] Mena et al., 2009 [31] Uрпи-Sarda et al., 2012 [32]	772 90% BMI >25 55%	69 56%	12–52	RCT	High cardiovascular risk who fulfilled at least 1 of 2 criteria: type 2 diabetes or 3 or more CHD risk factors (current smoking, hypertension [blood pressure >140/90 mm Hg or treatment with antihypertensive drugs], LDL-cholesterol level ≥ 160 mg/dl [or treatment with hypolipidemic drugs], HDL-cholesterol level ≤ 40 mg/dl, BMI ≥ 25 kg/m ² , or a family history of premature CHD)	Instruction to increase the 14-item Mediterranean score [27]; Free: - 1 l/week olive oil; Free: 15 g walnuts, 7.5 g hazelnuts, 7.5 g almonds daily	Reduce all types of fat
Itsiopoulos et al., 2011 [33]	27 30.15 100%	47–77 41%	12	Cross-over	Well-controlled T2D	The majority of foods (70%) on the intervention diet were provided free to subjects: traditional cooked meals, traditional whole grain bread, olives, dried fruit, nuts, Greek coffee and herbal tea, and extra virgin olive oil. Subjects were consulted individually by an APD and were advised to consume 3 servings of fruits per day and were required to prepare and consume the remainder of the food choices in the two-week cycle menu, which included predominantly fresh salads and simple meals such as fried whitebait, fried calamari and asparagus omelette	The habitual diet was deemed to be a satisfactory control diet as only subjects with good metabolic control were recruited

(continued on next page)

Table 1 (continued)

Reference	Sample size, mean baseline BMI (kg/m ²) % diabetics	Mean age (yrs), female (%)	Duration (weeks)	Study design	Health status	MD intervention	Comparison group
Konstantinidou et al., 2010 [34]	90 25 0%	43 70%	12	RCT	Healthy On the basis of a physical examination and routine biochemical and hematological laboratory examinations	The dietitian gave personalized advice during a 30-min session to each participant following the traditional Mediterranean diets, with recommendations on the desired frequency of intake of specific foods. Participants were instructed to use olive oil for cooking and dressing; increase consumption of fruit, vegetables and fish; consume white meat instead of red or processed meat; prepare homemade sauce with tomato, garlic, onion, aromatic herbs and olive oil to dress vegetables, pasta, rice, and other dishes; and, for alcohol drinkers, moderate consumption of red wine	Participants were advised by a dietitian to maintain their habitual lifestyle
Mekki et al., 2010 [41]	40 26.2 0%	61 100%	12	RCT	Moderate chronic renal failure with a glomerular filtration rate of 60–89 ml/min and dyslipidemia triacylglycerols > 1.7 mmol/L and/or total cholesterol > 5 mmol/L	Increased intake of MUFA, PUFA and fibers. To achieve this objective, the subjects were asked to consume olive oil and nuts for seasonings, whole grains (50 g of bread at each meal, 250 g of cereal or starch once a day), fruits (once a day), vegetables (200 g twice daily) and fish (twice a week)	Not receive modified nutritional advice
Michalsen et al., 2006 [35]	101 26.6 7.5	59.4 23%	52	RCT	Established CAD as verified by coronary angiography were recruited at two hospitals after undergoing diagnostic coronary angiography, percutaneous coronary intervention or stationary treatment for CAD. All patients had a coronary angiography performed at the cardiological unit during the index hospital stay or by their cardiologists in the previous 3 months	>5 fruits and vegetables/day, emphasis on root and green vegetables, >2 portions fatty fish/week, whole grains, flaxseed and nuts recommended, 3 servings/week meat replaced by poultry, fish or vegetarian dishes, olive oil, canola oil mainly, walnut and flaxseed oil for some dishes, margarine discouraged, modest red wine consumption with meals	Written and less detailed informations about the dietary principles of the MD, and some general advice about stress reduction by means of leaflets that were mailed shortly after randomization
Panunzio et al., 2011 [36]	61 27.5 0%	55 57%	25	RCT	Healthy, non-smokers	MD-FOOD Anticancer Program, developed in accordance with procedural model for designing Theory-Based Nutrition Education; Whole grains, legumes, vegetables, fresh fruit, dried fruit, oleaginous seeds, fish, cruciferous vegetables, liliaceous vegetables, soy, spices, green tea, berries, omega-3, soft fruit, tomato, citrus fruit	Habitual diet, and no contact with the intervention team
Poulsen et al., 2013 [42]	181 30.2 0%	41.9 71%	26	RCT	Waist circumferences ≥80 cm for women and ≥94 cm for men. one or more of the following conditions: plasma triglyceride concentrations ≥1.7 mmol/L, HDL-cholesterol concentrations ≤1.03 mmol/L for men and ≤1.29 mmol/L for women, hypertension (systolic/diastolic blood pressure >130/85 mm Hg), and impaired fasting glucose	New Nordic Diet: The basis of the diet is comprised of the following 15 food groups: fruit and vegetables (especially berries, cabbages, root vegetables, and legumes), potatoes, fresh herbs, plants and mushrooms gathered from the wild, nuts, whole grain, meats from livestock and game, fish and shellfish, and seaweed	The macronutrient composition and food groups of the control group were designed to match the diet that is commonly eaten in the adult Danish population aged 15–75 y and as defined by the latest survey of dietary habits in Denmark performed in 2003–2008
Sexton et al., 2013 [37]	23 25.8 0%	39.1 74%	12	RCT	Symptomatic asthma: by physician diagnosis and by at least one of the following: 12% post-bronchodilator increase in forced expiratory volume in 1 s or forced vital capacity; 20% increase in post-bronchodilator peak expiratory flow rate; or 15% morning PEF variability during the run-in period	Panagiotakos et al. MD score: non-refined cereals (whole grain bread and pasta, brown rice, etc.), fruit, vegetables, legumes, potatoes, fish, meat and meat products, poultry, full fat dairy products (like cheese, yoghurt, milk), as well as olive oil and alcohol intake who were encouraged to adopt MD and received intensive initial advice and 41-h consultation sessions with a dietitian	Who were offered one session with a dietitian, recipes, and free food at the end of the trial

Skoldstam et al., 2003 [38]	51 27 0%	58.5 80%	12	RCT	Rheumatoid Arthritis (1987 American College of Rheumatology criteria), disease duration of at least two years, clinically the disease must have been characterized as stable and under adequate control as assessed and documented by the patient's own rheumatology specialist at the latest consultation before the trial	Both olive oil and canola (rapeseed) oil were allowed for food preparation, baking, and in salad dressings. The patients were provided with a liquid margarine (80% fat) for cooking and a spreadable margarine (40% fat) to use on bread, both of which were based on canola oil. To suit Swedish subjects we adjusted the MD in certain aspects. The Cretan diet contains only small amounts of dairy products, predominantly yoghurt and cheese, whereas Swedes in general consume higher amounts of milk, fermented milk, and other dairy products. To eliminate the difference in consumption of dairy fats, our MD patients were asked to reduce the amounts of these food items or to choose low fat dairy products. No recommendations were given about alcohol consumption. To compensate for the polyphenols present in wine, we encouraged the MD group to drink green or black tea. To promote good compliance, an MD from the hospital canteen was served to our MD patients for the first three weeks. During these weeks each group of MD patients had six lessons from a dietician about Mediterranean food and cooking	The control patients were served ordinary hospital food during the outpatient based rehabilitation programme. For the rest of the study they were asked not to experiment by themselves, but to return to their usual diets
Sondergaard et al., 2003 [39]	131 n.d n.d	62.5 30%	52	RCT	Documented IHD TC: ≥ 193 mg/dl IHD was considered present when ≥ 1 of the following conditions were present: 1) recent/remote myocardial infarction, defined as the presence of at least 2 of the following: typical chest discomfort, transient elevation of creatine kinase level > 210 IU/L and creatine kinase MB > 20 IU/L, or persistent ST elevations or new pathologic Q waves in at least 2 leads; 2) unstable angina (UAP), defined as typical chest discomfort and dynamic ischemic changes in the electrocardiogram; and/or 3) stable angina pectoris (AP), defined as typical chest pain induced by physical exertion and positive exercise test results (horizontal or descending ST depressions > 1 mm measured 60 ms after the J-point accompanied by chest pain)	> 600 g of fruits and vegetables daily, reduce intake of fat, ≥ 1 x fish/week, eat bread and cereals, replace refined, hard, animal margarine products with canola oil	Offered booklets about heart-healthy diets Offered a single visit to a dietitian
Uusitupa et al., 2013 [40]	189 31.65 0%	54.5 66%	18–24	RCT	Metabolic syndrome BMI $27–38$ kg m ² and two other of IDF's criteria for MetS, fasting plasma glucose ≤ 7.0 mmol/L and a 2-h glucose value < 11.1 mmol/L at baseline detected by an oral glucose tolerance test	The main emphasis was on food items such as whole-grain products, abundant use of berries, fruit and vegetables, rapeseed oil, three fish meals per week, low-fat dairy products and avoidance of sugar-sweetened products. Intervention group received: whole-grain products, for example, various breads, berry products as frozen berries (e.g. strawberries, black currants, bilberries) and dried powder as well as dietary fats including rapeseed oil- and vegetable oil-based spreads. Local fruits (not provided) were mostly apples, pears and plums. Furthermore, either fish was directly provided or the expenses for consuming fish were covered to the study participants	Habitual Nordic diet

Abbreviations: ADP, Accredited Practicing Dietitian; BMI, Body-Mass-Index; CAD, coronary artery disease; CHD, coronary heart disease; *d*, daily; DASH, dietary approach to stop hypertension; DBP, diastolic blood pressure; GI, glycemic index; IDF, International Diabetes Federation; IFG, impaired fasting glucose; IHD, ischemic heart disease; MD, Mediterranean diet; MetS, metabolic syndrome; MI, myocardial infarction; MUFA, monounsaturated fatty acids; n.d., no data; PUFA, polyunsaturated fatty acids; RCT, randomized controlled trial; RDA, recommended daily allowance; SBP, systolic blood pressure; SFA, saturated fatty acids; T2D, type 2 diabetes; TC, total cholesterol.

Table 2 Pooled estimates of effect size (95% confidence intervals) expressed as weighted mean difference for the effects of MD vs. control intervention diets on outcomes of inflammation and endothelial function.

Outcomes	No. of studies	Sample size	MD	95% CI	p-values	Inconsistency I^2	Egger test Begg's test
CRP (mg/l)	14	1942	−0.98	[−1.48, −0.49]	<0.0001	91%	0.945 0.547
IL-6 (pg/ml)	6	1077	−0.42	[−0.73, −0.11]	0.008	81%	0.480 0.851
AD (μg/ml)	2	286	1.69	[0.27, 3.11]	0.02	78%	/
FMD (%)	2	210	1.86	[0.23, 3.48]	0.02	43%	/
ICAM-1 (ng/ml)	2	586	−23.73	[−41.24, −6.22]	0.008	34%	/
E-Selectin (ng/ml)	2	161	−0.67	[−6.51, 5.16]	0.82	20%	/

Abbreviations: AD, adiponectin; CRP, high sensitive C-reactive protein; FMD, flow mediated dilatation; ICAM-1, Inter cellular Adhesion Molecule 1; IL-6, Interleukin 6; VCAM-1, Vascular Adhesion Molecule 1.

CRP is an independent risk factor for CVD and CHD [43]. IL-6 is generally accepted as an acute phase inflammatory reactive protein produced upon stimulation by TNF- α and IL-1 [44], and a known trigger of CRP synthesis [45,46]. Chronic elevation of IL-6 levels was shown to be associated with increased CHD risk, although causality remains uncertain [47].

AD is regarded to have anti-inflammatory, anti-atherogenic, and insulin sensitizing functions [48]. However, inconclusive data regarding an association between elevated AD levels and risk reduction of CHD events, CVD as well as stroke, have been reported in recent systematic reviews and meta-analyses [49–51]. Endothelial dysfunction has been recognized as an early pathophysiologic event during a variety of CVD including atherosclerosis [52]. Evidence from epidemiological studies and clinical trials suggested that impairment of endothelial function is an independent risk factor for the incidence of cardiovascular events in adults [53]. Results of a meta-regression including 11,984 subjects indicated that in populations at low risk of CVD, FMD is a strong cardiovascular risk factor regarding the estimated 10-year risk of CHD [54]. A recent meta-analysis suggested a 13% increase in the risk of future CVD events for every 1% decrease in FMD [13]. By transferring these data to the findings of the present meta-analysis, reduction in CVD risk following an MD will be approximately 24% stronger as compared to the respective control groups. Esposito et al. established their own endothelial function score observing significant improvements in the MD group compared with the controls [27]. Furthermore results of short-term MD dietary intervention trials showed beneficial effects on markers of endothelial function [55,56].

Interconnections between MD and markers of inflammation as well as endothelial function have been suggested by other studies as well. The Nurses' Health Study and the ATTICA-study reported evidence of a correlation between higher adherence to MD and reductions in IL-6, CRP, TNF- α , ICAM-1, s-VCAM-1, E-Selectin, fibrinogen, homocysteine levels, and white blood cell count [57,58], and a cross-sectional analysis of the PREDIMED trial showed that the upper tertiles of consumption of cereals, fruit, nuts, and virgin olive oil tended to have lower serum concentrations of IL-6, CRP, VCAM-1, and ICAM-1 [59].

There is a causal link between oxidative stress, inflammation, endothelial dysfunction, and CVD/CHD [60]. Inflammatory processes promote the production of reactive oxygen species which in turn interfere with transcriptional factors such as NF- κ B, cytokines as well as other mediators of a pro-inflammatory status like activator protein-1, VCAM-1, and ICAM-1 [61]. Taken together, this promotes the development of endothelial dysfunction and apoptosis [62]. Chronic low-grade inflammation might be involved in insulin resistance and endothelial dysfunction, providing a model that links obesity with atherosclerosis, the main cause of cardiovascular disease and mortality. With respect to the potential impact of MD on oxidative stress, the LIPGENE study recently showed that a diet high in MUFA enhances postprandial glutathione levels and the glutathione/oxidized glutathione ratio compared with two types of LF diets and a diet high in SFA. Furthermore, the MUFA-rich diet decreased the postprandial inflammatory state as compared to the SFA-rich diet [63,64]. Postprandial levels of oxidative stress were shown to play a major role in the development of atherosclerosis [65].

One explanation for the protective mechanisms in the prevention of atherosclerotic vascular disease of MD is provided by its main component virgin olive oil [66]. At least *in vitro*, polyphenols from virgin olive oil suppressed the reactive oxygen species-mediated activation of NF- κ B-dependent as well as the consecutive expression of matrix metalloproteinase-9 and cyclooxygenase-2, thereby reducing tube formation and migration [67].

Limitations

The findings of the present meta-analysis with respect to AD, FMD, ICAM-1, and E-selectin should be interpreted conservatively, since only two trials reported on the outcome of these parameters. Another major limitation often found in nutritional intervention trials is the heterogeneity of the study protocols. The RCTs included in the present analyses varied with regard to type(s) of diets used (energy restriction, isocaloric), definitions of MD, study population, intervention time (12–104 weeks), as well as assessment methods to validate dietary intakes. Various heterogeneous patterns of MD focusing on a specific type

of a diet as a whole rather than on individual diet components were effective in reducing inflammatory and vascular markers. Following subgroup analyses, the beneficial effects of an MD turned out to be more prominent (CRP and IL-6) in trials with change from baseline values, long-term, low risk of bias (only CRP), larger sample size, and non-obese subjects. The subgroup data for IL-6 should be interpreted with caution, since only six trials could be pooled for the respective analysis. Only few studies gave detailed information on the quality of their respective setup (e.g. method of randomization, follow-up protocol with reasons for withdrawal, see [Supplemental Fig. S1](#)), again demanding a conservative interpretation of results. The definition of the MD dietary pattern is not homogeneous, and may vary across different geographical and cultural contexts. The components required to meet the definition of an MD dietary pattern were based on a Cochrane Review by Rees et al. [16], resulting in the inclusion of two trials which were not described as an MD [24,25].

In conclusion, the present systematic review provides evidence that a Mediterranean dietary pattern is associated with reductions of markers of inflammation and endothelial dysfunction. However, findings for AD, FMD, ICAM-1, and E-selectin were based on combination of only two studies, thus requiring a conservative interpretation. The meta-analysis adds scientific evidence that MD decreases inflammation and improves endothelial function, providing a causal link for findings of observational studies reporting a significant association between adherence to MD and reduced risk of mortality and CVD/CHD.

Conflict of interest

The authors declare to have no conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.numecd.2014.03.003>.

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Review Article

Adherence to a Mediterranean diet and risk of diabetes: a systematic review and meta-analysis

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Abstract

Objective: Adherence to a Mediterranean diet is associated with significant improvements in health status. However, to date no systematic review and meta-analysis has summarized the effects of Mediterranean diet adherence on the risk of type 2 diabetes mellitus.

Design: Electronic searches for randomized controlled trials and cohort studies were performed in MEDLINE, SCOPUS, EMBASE and the Cochrane Trial Register until 2 April 2014. Pooled effects were calculated by an inverse-variance random-effect meta-analysis using the statistical software Review Manager 5.2 by the Cochrane Collaboration.

Setting: Meta-analysis of randomized controlled trials and cohort studies.

Subjects: Eligibility criteria: 19+ years of age.

Results: One randomized controlled trial and eight prospective cohort studies (122 810 subjects) published between 2007 and 2014 were included for meta-analysis. For highest *v.* lowest adherence to the Mediterranean diet score, the pooled risk ratio was 0.81 (95 % CI 0.73, 0.90, $P < 0.0001$, $I^2 = 55\%$). Sensitivity analysis including only long-term studies confirmed the results of the primary analysis (pooled risk ratio = 0.75; 95 % CI 0.68, 0.83, $P < 0.00001$, $I^2 = 0\%$). The Egger regression test provided no evidence of substantial publication bias ($P = 0.254$).

Conclusions: Greater adherence to a Mediterranean diet is associated with a significant reduction in the risk of diabetes (19 %; moderate quality evidence). These results seem to be clinically relevant for public health, in particular for encouraging a Mediterranean-like dietary pattern for primary prevention of type 2 diabetes mellitus.

Keywords
Mediterranean Diet
Meta-analysis
Diabetes risk
Systematic review

The worldwide incidence of people with type 2 diabetes mellitus (T2DM) is projected to rise from 171 million in 2000 to 380 million in 2025, causing enormous costs for health-care systems⁽¹⁾. The typical Mediterranean diet (MD), which was first postulated by Ancel Keys in the 1960s⁽²⁾, is characterized by high intakes of MUFA, vegetables and fruits, plant proteins, whole grains, fish and low-fat dairy products, moderate alcohol (red wine) intake, and low red meat consumption⁽³⁾. In the Seven Countries Study, Keys reported the beneficial effects of an MD on human health⁽²⁾. Since then, dietary pattern analysis has been widely used to detect the cumulative effects of diet on health. T2DM is a major chronic disease originating from different genetic, behavioural and

environmental risks⁽⁴⁾, with impaired glucose tolerance being an indicator for the development of a diabetic metabolic state⁽⁵⁾. Lifestyle intervention studies in randomized trial settings have demonstrated a reduction in the relative risk to develop T2DM of 30–67 %⁽⁶⁾ with sustainable long-term reduction of incidence^(7,8), although a need to develop standardized lifestyle interventions may be necessary to enhance their efficacy⁽⁹⁾. MD is a homogeneous and straightforward construct having its roots in southern European eating patterns. Adherence to an MD is associated with significant improvements in health status ranging from reduced risk of mortality to neurodegenerative diseases^(10,11). However, to date no systematic review and meta-analysis has summarized the effects of

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MD adherence on the risk of T2DM. Therefore, the aim of the present study was to meta-analyse randomized controlled trials and cohort studies that have assessed the effect of adherence to an MD on the risk of T2DM.

Methods

Literature search

Literature searches were performed using the electronic databases MEDLINE, SCOPUS, EMBASE and the Cochrane Trial Register until 2 April 2014 using the following keywords: 'Mediterranean diet AND diabetes'. Moreover, the reference lists from retrieved articles were checked to search for further relevant studies, and systematic reviews and meta-analysis were searched by two authors. The literature search as well as article abstraction was conducted independently by two authors (L.S. and B.M.). Relevance of studies was assessed with a hierarchical approach (title, abstract and full manuscript). After an initial screening of titles and abstracts, studies included by both reviewers were compared and disagreement was resolved by consensus.

Eligibility criteria

Studies were included in the meta-analysis if they met all of the following criteria: (i) randomized controlled trial or cohort study design; (ii) reported adjusted relative risks (RR) for T2DM with corresponding 95 % confidence intervals; (iii) evaluated the association of an *a priori* score used for assessing adherence to an MD; and (iv) when a study seemed to have been published in duplicate, the version containing the most comprehensive information was selected. Studies including pregnant women were excluded, as were case-control studies, cross-sectional studies and studies that were not published as original reports (such as conference abstracts, letters to the editor without original data, commentaries and reviews).

Quality assessment

Full copies of studies were independently assessed for methodological quality by two authors (L.S. and B.M.) using the Jadad score for randomized controlled trials and the Newcastle Ottawa Scale for cohort studies^(12,13) (Table 1).

Data extraction

Two investigators (L.S. and B.M.) independently collected the relevant reports using a standardized form. The following data were extracted from each study: (i) first author's last name; (ii) year of publication; (iii) country of origin; (iv) sample size; (v) study length; (vi) age at entry; (vii) sex; (viii) T2DM diagnosis criteria; (ix) study design; (x) components of the MD score; (xi) adjustment factors; (xii) quality score; (xiii) dietary assessment method; and (xiv) risk estimates with their corresponding 95 % confidence intervals. When a study provided several risk estimates, the maximally adjusted models were chosen.

Statistical analysis

The meta-analysis was performed with the adjusted RR of the highest compared with the lowest MD adherence score based on random-effects modelling according to DerSimonian and Laird incorporating both within- and between-study variability. To evaluate the weighting of each study, the SE for log(RR) of each study was calculated and regarded as the estimated variance of log(RR)⁽¹⁴⁾. Random-effects modelling with restricted maximum likelihood estimate, which is more appropriate if the number of included studies is small, was also used to confirm the final risk estimates. Heterogeneity was estimated by the Cochrane *Q* test together with the *I*² statistic. An *I*² value >50 % indicates substantial heterogeneity across studies⁽¹⁵⁾. Funnel plots were used to assess potential publication bias. To determine the presence of publication bias, the symmetry of the funnel plots in which mean differences were plotted against their corresponding SE was assessed. In addition, the Egger test was performed to test for potential publication bias⁽¹⁶⁾.

Results

Literature search

Nine papers extracted from 1408 articles met the eligibility criteria and were included in the quantitative analysis^(17–25). One cohort study was excluded since the primary outcome was the risk of gestational diabetes⁽²⁶⁾. The detailed steps of the meta-analysis article selection process are described as a flow diagram in Fig. 1.

Study characteristics

One randomized controlled trial and eight prospective cohort studies (sample size: 122 810 subjects) published between 2007 and 2014 were included for meta-analysis. Study duration plus follow-up ranged between 3.2 and 20 years. All cohort studies used an FFQ for dietary assessment. General study characteristics are summarized in Table 1.

To assess the quality of the included studies, a 9-point scoring system was used according to the Newcastle Ottawa Scale. A score of ≥7 points was defined as high study quality study (see online supplementary material, Table S1); all cohort studies were of high quality. The cut-offs for the high *v.* low MD score differed between the studies (three cohorts used quintiles^(17,18,20), two studies used quartiles^(23,26) and tertiles^(19,22), and one study used cluster analysis⁽²¹⁾).

Mediterranean diet and risk of diabetes

The pooled RR for the highest *v.* lowest adherence to the MD score was 0.81 (95 % CI 0.73, 0.90, *P* < 0.0001, *I*² = 55 %; Fig. 2). Stratified RR are summarized in Table S2 (see online supplementary material). For studies with a long-term follow-up (≥10 years), an RR of 0.75 (95 % CI 0.68,

Table 1 General characteristics of the studies included in the present meta-analysis

Authors, year and reference	Country and cohort name	Study type	Outcome and criteria	Population and health status	Follow-up (years)	Age at entry (years)	Sex	Components of MD score or dietary intervention	Adjustments	Quality score
Abiemo <i>et al.</i> (2013) ⁽¹⁷⁾	USA Multi-Ethnic Study of Atherosclerosis (MESA)	Cohort	T2DM FPG < 7.0 mmol/l; fasting serum insulin levels	<i>n</i> 5390 At the baseline exam free of clinical CVD	6	45–84	M/W	(i) ↑ Vegetables (except potatoes); (ii) ↑ legumes; (iii) ↑ fruit and fruit juices; (iv) ↑ nuts; (v) ↑ whole grain products; (vi) ↑ fish; (vii) ↓ red meat and processed meats; (viii) ↑ MUFA:SFA; (ix) ↓ dairy products than median intake	Age, sex, race/ethnicity, educational level, family income, PA, smoking status, total energy intake, WC	7
Brunner <i>et al.</i> (2008) ⁽²¹⁾	England Whitehall II	Cohort	T2DM Self-report, diabetic medication, OGTT	<i>n</i> 7731 At the baseline exam free of clinical CVD	15	35–69	M/W	(i) ↑ Wholemeal bread; (ii) ↑ fruits; (iii) ↑ vegetables; (iv) ↑ pasta and rice; (v) ↑ wine; (vi) ↓ intake of full-cream milk; (vii) ↑ butter; (viii) moderate white bread	Age, sex, ethnicity, dietary energy misreporting, social position, smoking status, leisure-time PA	7
De Koning <i>et al.</i> (2011) ⁽¹⁸⁾	USA Health Professionals Follow Up (HPFS)	Cohort	T2DM Self-report medication use or positive glucose test (until 1998: NDDG criteria; after 1998: ADA criteria; 97 % of the cases were validated by medical record)	<i>n</i> 41 615 At the baseline exam free of clinical CVD	≤20	n.d.	M	(i) ↑ Vegetables (no potatoes); (ii) ↑ legumes; (iii) ↑ fruits; (iv) ↑ nuts; (v) ↑ whole grains; (vi) ↑ fish; (vii) ↑ MUFA: SFA; (viii) ↓ red meat and processed meats; (ix) moderate alcohol intake	Age, smoking, PA, coffee intake, family history of T2DM, BMI, total energy	9
Salas-Salvado <i>et al.</i> (2014) ⁽²⁵⁾	Spain Prevencion con dieta mediterranea trial (PREDIMED)	RCT	T2DM OGTT and FPG < 7.0 mmol/l	<i>n</i> 3541 At the baseline exam free of clinical CVD. At least three of the following CVD risk factors: smoking, hypertension, dyslipidaemia, overweight, family history of premature CVD	4.1	55–80	M/W	(i) ↑ Olive oil; (ii) ↑ fruits, vegetables, legumes and fish; (iii) ↓ total meat, recommending white meat instead of red or processed meat; (iv) homemade tomato sauce; (v) ↓ butter, cream, fast food, sweets, pastries, sugar-sweetened beverages; (vi) moderate red wine	Energy intake, BMI, WC, PA, smoking status, FPG, use of lipid-lowering drugs, MedDiet Score	4*

Table 1 Continued

Authors, year and reference	Country and cohort name	Study type	Outcome and criteria	Population and health status	Follow-up (years)	Age at entry (years)	Sex	Components of MD score or dietary intervention	Adjustments	Quality score
InterAct Consortium (2011) ⁽²²⁾	European Prospective Into Cancer and Nutrition (EPIC) (excluded: Greece, Norway)	Cohort	T2DM Medical record	<i>n</i> 16 154 At the baseline exam free of clinical CVD	4.4	25–70	M/W	(i) ↑ Vegetables (no potatoes); (ii) ↑ legumes; (iii) ↑ fruits; (iv) ↑ nuts; (v) ↑ whole grains; (vi) ↑ fish; (vii) ↑ MUFA: SFA; (viii) ↓ red meat and processed meats; (ix) moderate alcohol intake	Energy intake, sex, BMI, education level, PA, smoking status	9
Martínez-González <i>et al.</i> (2008) ⁽¹⁹⁾	Spain Seguimiento Universidad de Navarra (SUN)	Cohort	T2DM ADA (symptoms of diabetes + random plasma glucose concentration ≥ 11.1 mmol/l or FPG ≥ 7.0 mmol/l, OGTT)	<i>n</i> 13 380 At the baseline exam free of clinical CVD	3.2	20–29	M/W	(i) ↑ Vegetables; (ii) ↑ legumes; (iii) ↑ fruits; (iv) ↑ nuts; (v) ↑ whole grains; (vi) ↑ fish; (vii) ↑ MUFA:SFA; (viii) ↓ meat and meat products; (ix) moderate milk and dairy products; (x) moderate alcohol intake	Age, sex, total years of university education, BMI, family history of diabetes, hypertension at baseline, PA, hours sitting down per week, smoking, total energy intake	8
Mozaffarian <i>et al.</i> (2007) ⁽²⁰⁾	Italy Gruppo Italiano per lo Studio della sopravvivenza nell'infarto miocardico (GISSI)	Cohort	T2DM New use of diabetic medication or FPG ≥ 7.0 mmol/l	<i>n</i> 8291 With MI	11.34	20–90	M/W	(i) ↑ Cooked and raw vegetables; (ii) ↑ fruits; (iii) ↑ fish; (iv) ↑ olive oil; (v) moderate alcohol intake	Age, sex, smoking status, time from MI to enrolment, treatment assignment, BMI, maximum exercise tolerance during stress testing, ischaemia during stress testing, NYHA heart failure symptoms, CCS angina symptoms, history of hypertension, prior MI previous to index MI, ACE inhibitor use, β -blocker use, diuretic use, lipid-lowering medication use, consumption of cheese, wine and coffee	7

Table 1 Continued

Authors, year and reference	Country and cohort name	Study type	Outcome and criteria	Population and health status	Follow-up (years)	Age at entry (years)	Sex	Components of MD score or dietary intervention	Adjustments	Quality score
Rossi <i>et al.</i> (2013) ⁽²³⁾	European Prospective Into Cancer and Nutrition (EPIC) (only Greek cohort)	Cohort	T2DM Self-report, medical record, diabetic medication use	n 22 295 At the baseline exam free of clinical CVD	4	20–80	M/W	(i) ↑ Vegetables; (ii) ↑ legumes; (iii) ↑ fruits; (iv) ↑ nuts; (v) ↑ cereals; (vi) ↑ fish and seafood; (vii) ↑ MUFA:SFA; (viii) ↓ meat and meat products; (ix) ↓ dairy products; (x) 10–50 g ethanol/d for men and 5–25 g ethanol/d for women considered as moderate alcohol consumption	Age, sex, level of education, PA, BMI, WHR, total energy intake	9
Tobias <i>et al.</i> (2012) ⁽²⁴⁾	USA Nurses' Health Study II (NHS II)	Cohort	T2DM One or more classic symptoms (i.e. excessive thirst, polyuria, etc.) and FPG <7.7 mmol/l (after 1998: <7.0 mmol/l) or random plasma glucose level ≥11 mmol/l	n 4413 With prior GDM	14	24–44	W	(i) ↑ Vegetables; (ii) ↑ legumes and soya; (iii) ↑ fruits; (iv) ↑ nuts; (v) ↑ whole grain products; (vi) ↑ fish and seafood; (vii) ↑ MUFA:SFA; (viii) ↓ red meat and processed meats; (ix) moderate alcohol intake	Age, total energy intake, age at first birth, parity, ethnicity, parental diabetes, oral contraceptive use, menopause, smoking status	8

MD, Mediterranean diet; RCT, randomized controlled trial; FPG, fasting plasma glucose; OGGT, oral glucose tolerance test; NDDG, National Diabetes Data Group; ADA, American Diabetes Association; MI, myocardial infarction; GDM, gestational diabetes mellitus; n.d., not determined; M, men, W, women; PA, physical activity; WC, waist circumference; NYHA, New York Heart Association; CCS, Canadian Cardiovascular Society; ACE, angiotensin-converting enzyme; WHR, waist:hip ratio.

ADA criteria: symptoms of diabetes plus casual plasma glucose concentration ≥ 11.1 mmol/l or FPG ≥ 7.0 mmol/l, or 2 h post-load glucose ≥ 11.1 mmol/l during an OGGT; in the absence of unequivocal hyperglycaemia.

*Jadad score.

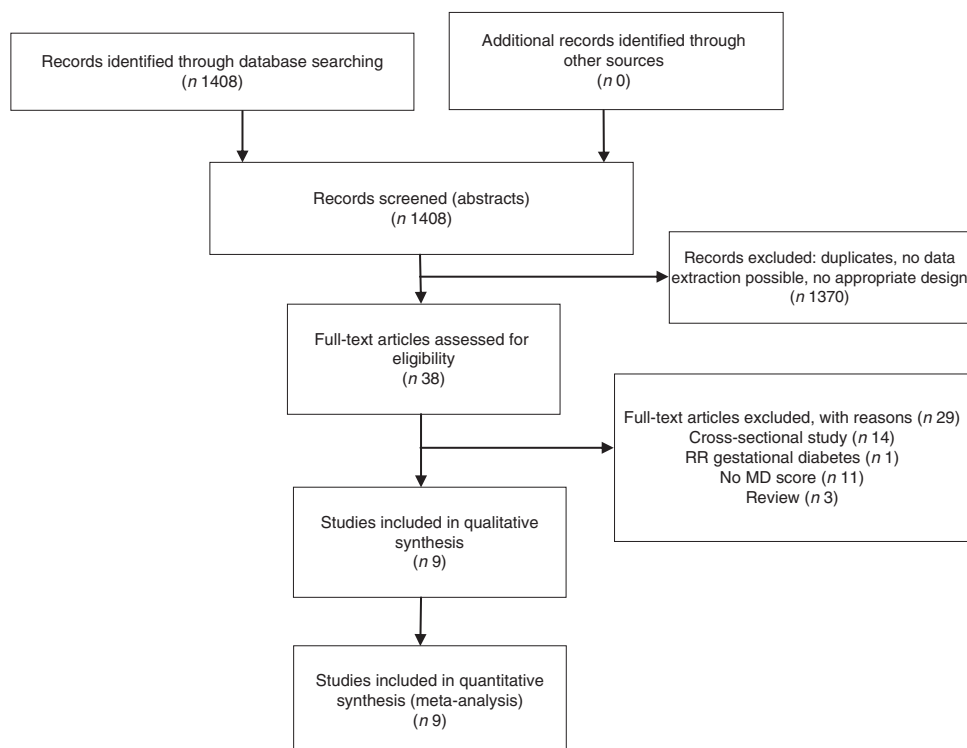


Fig. 1 Flow diagram showing detailed steps of the article selection process for the present meta-analysis (RR, relative risk; MD, Mediterranean diet)

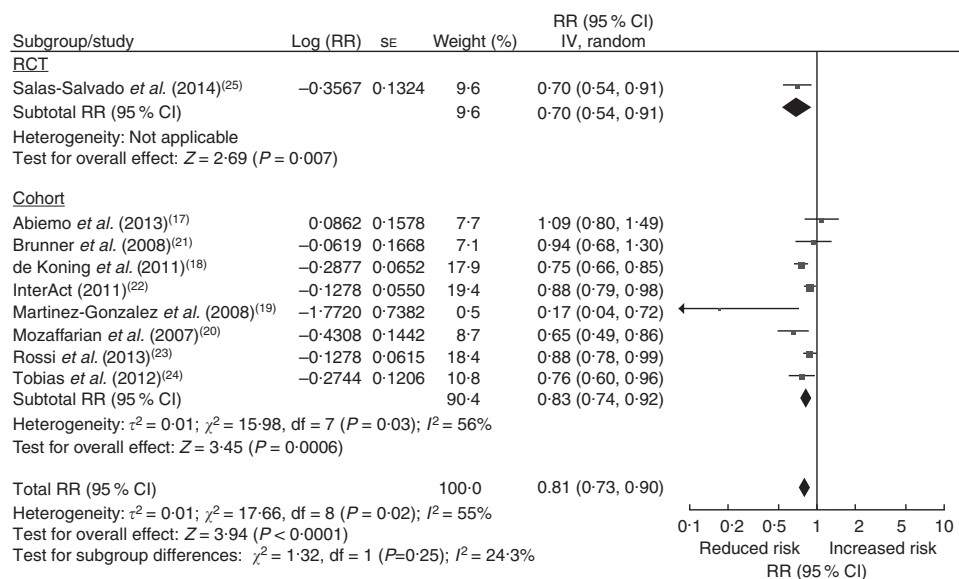


Fig. 2 Forest plot showing the relative risk (RR) and 95% confidence interval of type 2 diabetes for the highest v. lowest adherence to a Mediterranean diet score for one randomized controlled trail (RCT) and eight prospective cohort studies (122 810 subjects) published between 2007 and 2014. For each study, the black square indicates the RR, the size of which indicates the study's weight in the analysis (weights are from random-effects analysis) and the horizontal line represents the 95% CI. The centre of the diamond indicates the summary estimate of the RR and its width represents the 95% CI of the summary RR estimate

0.83, $P < 0.00001$, $I^2 = 0\%$) was found, and for large sample size ($\geq 10\,000$), an RR of 0.82 (95% CI 0.72, 0.94, $P = 0.005$, $I^2 = 67\%$) could be observed. There was a significant association in the European analysis (RR = 0.81; 95% CI

0.71, 0.93, $P = 0.002$) but not in the US analysis (RR = 0.82; 95% CI 0.68, 1.00, $P = 0.05$). Exclusion of studies by Tobias *et al.*⁽²⁴⁾ (history of gestational diabetes) and Mozaffarian *et al.*⁽²⁰⁾ (secondary prevention of CVD) confirmed the

results of the main analysis ($RR=0.84$; 95 % CI 0.75, 0.94, $P=0.003$, $I^2=57\%$). Since cut-off and MD scores varied between studies, we performed a sensitivity analysis investigating the association between a 2-point increase of adherence to the MD score (defined by Trichopoulou⁽²⁷⁾) and the risk of T2DM. Six of nine^(17–19,22,23,26) studies were included and showed a significant risk reduction ($RR=0.93$; 95 % CI 0.89, 0.98, $P=0.007$, $I^2=69\%$; see online supplementary material, Fig. S1).

Publication bias

The funnel plots for diabetes incidence indicated low asymmetry (with the exception of one outlier), suggesting that the risk of publication bias is low (see online supplementary material, Fig. S2). The Egger test for small study effects yielded non-significant results ($P=0.254$).

Discussion

The present meta-analysis including >100 000 subjects revealed a significant association between adherence to dietary patterns exhibiting specific MD characteristics and decreased risk of T2DM. The overall quality of evidence rated according to the GRADE guidelines for the main outcome was moderate⁽²⁸⁾. To the best of our knowledge, the present study represents the first meta-analysis showing a possible association between adherence to an MD and risk of T2DM.

With respect to potential mechanisms of action, there seems to be a causal link between oxidative stress, inflammation, endothelial dysfunction and diabetes⁽²⁹⁾. Interconnections between MD and markers of inflammation/endothelial function have been suggested by a recent meta-analysis of intervention trials⁽³⁰⁾. Different adherence scores measuring compliance to an MD were used in epidemiological studies since Keys' first findings, and one of the most relevant scores was established by Trichopoulou *et al.*⁽²⁷⁾. Several studies of the present meta-analysis used MD scores based on Trichopoulou *et al.* with only slight variations. The combination of components and the adherence to the whole concept of an MD seems pivotal: increasing fruit and vegetable intake as a sole option only showed weak overall effects on risk for developing T2DM⁽³¹⁾. Making use of synergistic effects of different nutrient compounds especially in an MD has shown to be more distinct and effective. Thus, compared with targeting single nutrients on their health impact, dietary pattern analysis is especially useful in a public health context providing clear-cut messages for stakeholders.

In our meta-analysis, the relative risk for developing T2DM when adhering to an MD was significantly different when comparing European and US studies. Risk for T2DM development was significantly more decreased in European studies as compared with their US counterparts. This finding may be due to confounders that were

not assessed in the various studies. Subgroup analysis including studies of great population (sample size $\geq 10\,000$) and long-term follow-up (≥ 10 years) resulted in a more pronounced effect of MD adherence on T2DM ($RR=0.82$, $P=0.005$, $I^2=67\%$ and $RR=0.75$, $P<0.00001$, $I^2=0\%$) when compared with studies with a smaller sample size ($<10\,000$) or short-term studies (<10 years). Especially long-term follow-up showed a prominent and significant decrease of T2DM risk of 25 %, suggesting a beneficial long-term effect of MD on T2DM risk.

There are some limitations to our study that should be considered. First, although most studies used a stringent MD score computation based on Trichopoulou *et al.*⁽²⁷⁾, a more homogeneous MD score computation would be desirable to make studies even more comparable. Second, two studies with a slightly different study and population design (Mozaffarian *et al.*, secondary prevention of CVD⁽²⁰⁾; Tobias *et al.*, volunteers with history of gestational diabetes⁽²⁴⁾) were included in the present meta-analysis. Thus, all subjects in these studies had a history of a disease, which may be associated with higher diabetes risk than for healthy subjects. However, results of the primary analysis could be confirmed following exclusion of the respective studies. High inter-study heterogeneity most likely results in a reduction in reliability of estimates. Since considerable heterogeneity could be observed for the primary analysis in the present systematic review, additional sensitivity analyses were performed to evaluate whether this could have been due to a single study. However, no single study seemed to have a considerable effect on heterogeneity. Furthermore, meta-analyses are limited by the included study types and cohort studies are at risk of various potential sources of bias. For example, response bias as well as errors in the assessment of diets is a persistent problem when analysing associations between diet and disease.

Conclusion

In conclusion, the current systematic review and meta-analysis shows that adherence to an MD is associated with a decreased risk of becoming diabetic at a reasonable magnitude (19 %). Overall, the present study supports the relevancy and effectiveness of a long-term adherence to an MD as an important measure in the primary prevention of T2DM.

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results, drafted the manuscript and finalized the manuscript. All authors read and approved the final manuscript. *Ethics of human subject participation:* Ethical approval was not required.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S1368980014001542>

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Adherence to Mediterranean diet and risk of cancer: A systematic review and meta-analysis of observational studies

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The aim of this research study was to meta-analyze the effects of adherence to Mediterranean diet (MD) on overall cancer risk, and different cancer types. Literature search was performed using the electronic databases MEDLINE, SCOPUS and EMBASE until January 10, 2014. Inclusion criteria were cohort or case-control studies. Study specific risk ratios (RRs) were pooled using a random effect model by the Cochrane software package Review Manager 5.2. Twenty-one cohort studies including 1,368,736 subjects and 12 case-control studies with 62,725 subjects met the objectives and were enclosed for meta-analyses. The highest adherence to MD category resulted in a significantly risk reduction for overall cancer mortality/incidence (cohort; RR: 0.90, 95% CI 0.86–0.95, $p < 0.0001$; $I^2 = 55\%$), colorectal (cohort/case-control; RR: 0.86, 95% CI 0.80–0.93, $p < 0.0001$; $I^2 = 62\%$), prostate (cohort/case-control; RR: 0.96, 95% CI 0.92–0.99, $p = 0.03$; $I^2 = 0\%$) and aerodigestive cancer (cohort/case-control; RR: 0.44, 95% CI 0.26–0.77, $p = 0.003$; $I^2 = 83\%$). Nonsignificant changes could be observed for breast cancer, gastric cancer and pancreatic cancer. The Egger regression tests provided limited evidence of substantial publication bias. High adherence to a MD is associated with a significant reduction in the risk of overall cancer mortality (10%), colorectal cancer (14%), prostate cancer (4%) and aerodigestive cancer (56%).

According to the World Health Organization in 2008, 7.6 million people (representing 13% of all causes of death) worldwide died owing to malignant neoplasm, a figure predicted to rise to 12 million deaths from cancer by 2030. The most common cancer is breast cancer, followed by colorectal and lung cancer.¹ Historically, the incidence of overall cancer was lower in Mediterranean countries compared to northern countries, the United Kingdom and the United States.² The typical Mediterranean diet (MD) as postulated first by Ancel Keys in the 1960³ is characterized by a high intake of monounsaturated fatty acids (MUFAs) derived from olive oil, vegetables and fruits, plant proteins, whole grains, fish, low-fat dairy, moderate alcohol (red wine) intake and low red meat consumption.⁴ Data from the Seven Countries Study reported by Keys *et al.* emphasized the beneficial effects of an MD on human health.³ Usually, an MD is composed of the following food groups: legumes, cereals, fruits/nuts, vegetables, extra-virgin olive oil, red wine in moderate amounts and low amounts of red meat, poultry and dairy products.⁵ The main component of olive

oil as used in MD is oleic acid accompanied by other lipophilic compounds such as alpha-tocopherol and phenols with strong anti-oxidant and anti-inflammatory effects as well as a potential influence on cell proliferation, cell cycle progression, apoptosis and arachidonic acid metabolism in cancer cells.⁶ However, one should note that only virgin and extra-virgin varieties retain the lipophilic components of the fruit, while common and refined olive oil contains only a fraction of the original amounts of these compounds.⁷

Fruits, vegetables, legumes and nuts, also representing regular ingredients of an MD, are good sources of dietary fiber as well as antioxidants and appear to be associated with a lower risk of different cancers types.⁸ In 2008, a meta-analysis of cohort studies provided convincing evidence that a 2-point increase of adherence to MD score conferred a significant protection against mortality, cardiovascular diseases and major chronic degenerative diseases such as cancer.⁹ The updated version of this meta-analysis could confirm the beneficial effects of an MD on overall incidence of cancer and cancer mortality.¹⁰ However, to our knowledge, no previous meta-analysis has analyzed the effects of an MD on different cancer types. Therefore, the aim of our study was to meta-analyze observational studies (cohort and case-control studies) that investigated the effects of adherence to MD on overall cancer risk, and risk of different cancer types (e.g., colorectal, breast, gastric, prostate, aerodigestive and pancreas).

Key words: Mediterranean diet, cancer, meta-analysis

Additional Supporting Information may be found in the online version of this article.

Conflict of interest: Nothing to report

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Methods

The review protocol has been registered in PROSPERO International Prospective Register of Systematic Reviews (crd.york.ac.uk/prospero/index.asp Identifier: CRD42013004382).

What's new?

Adherence to a “Mediterranean Diet” is associated with significant improvements in health status, including a lower overall risk of cancer. In this meta-analysis, the authors examined the impact of this type of diet on risks for specific types of cancer, as well as overall risk. They found that high adherence to a Mediterranean diet is associated with a significant reduction in the risk of several types of cancer, especially colorectal and aerodigestive cancers.

Data sources and searches

Queries of literature were performed using the electronic databases MEDLINE (until January 10, 2014), EMBASE (until January 10, 2014) and SCOPUS (until January 10, 2014) with no restrictions to calendar date using the following search terms: (“Mediterranean diet” or “diet” or “Mediterranean” or “dietary pattern” or “dietary adherence” or “dietary score”) in combination with (“cancer” or “neoplasm” or “neoplastic disease”) and (“prospective” or “follow-up” or “cohort”). The search strategy had no language restrictions. Moreover, the reference lists from retrieved articles were checked to search for further relevant studies. This systematic review was planned, conducted and reported in adherence to standards of quality for reporting meta-analyses of observational studies in epidemiology guidelines.¹¹ Literature search was conducted independently by both authors, with disagreements resolved by consensus.

Study selection

Studies were included in the meta-analysis if they met all the following criteria: (i) cohort or case-control study design; (ii) evaluation of the association of score used for assessing adherence to a MD and overall mortality/incidence from cancer, and/or different types of cancer; (iii) adjustment of relative risks (RRs), hazard ratios (HRs) or odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs) or the data necessary to calculate these; (iv) outcomes of interest include overall risk of mortality/incidence from cancer, risk of breast/colorectal/prostate/gastric/aerodigestive (esophageal/pharyngeal)/pancreatic cancer and (v) when a study appeared to have been published in duplicate, the version containing the most comprehensive information was selected.

Data extraction and quality assessment

The following data were extracted from each study: the first author's last name, year of publication, study origin, cancer type, sample size, number of cases and controls (case-control studies), study length (cohort studies), age at entry, sex, study design, components of MD score, adjustment factors, quality score and risk estimates (HR, RR and OR) (highest vs. lowest category) with their corresponding 95% CIs. If separate risk estimates for males and females were available in one study, we treated it as two separate studies. When a study provided several risk estimates, the maximally adjusted model was chosen. To assess the study quality, a 9-point scoring system according to the Newcastle-Ottawa Scale (NOS) was used.

Hence, the full score was 9, and a high-quality study in our analysis was defined as a study with ≥ 7 points.¹² The diagnosis of cancer was always supported by pathological examination of tissue samples.

Definition: Adherence to MD

Adherence to a MD was defined through scores that estimated the conformity of the dietary pattern of the studied population with the traditional Mediterranean dietary pattern. Specifically, people whose consumption of components considered to be part of an MD (olive oil, vegetables, fruits, legumes, cereals, fish and a moderate intake of red wine during meals) was above the median consumption of the population were assigned a value of 1/2, whereas a value of 0 was given to those with consumptions below the median. By contrast, people whose consumption of components presumed not to form part of a MD (red and processed meats and dairy products) was above the median consumption of the population had a value of 0 assigned, and the others had a value of 1. However, some differences among the studies existed, especially in relation to the food category of vegetables (grouped with potatoes in some studies),^{13–17} meat and meat products (grouped with poultry in several studies),^{18–32} and nuts and seeds (grouped with fruits in several studies,^{18–24,26–29,31–36} with legumes in two studies,^{17,25} and considered a group by themselves in some others^{37–44}) as well as milk and dairy products (not present in some studies^{30,37–43,45–47}), fish (not present in one study⁴⁸) and MUFA (not present in one study¹⁷). Furthermore four included studies used factor analysis^{16,30,36,45} and one study cluster analysis.³³ Thus, the total adherence scores (estimated as the sum of the above indicated scores of 0 and 1) varied from a minimum of ≤ 2 –6 points indicating the lowest adherence category to a maximum of ≥ 6 –11 points reflecting highest adherence category to a MD, in all studies, with the exception of four studies (Tables 1 and 2).^{30,45,46,49}

Statistical analysis

The meta-analysis was performed by combining the multi-variable adjusted RRs, HR or ORs of the highest compared with the lowest MD adherence category based on random effects model using Der Simonian-Laird method, which incorporated both within and between study variability. To evaluate the weighting of each study, the standard error for the logarithm HR/RR/OR of each study was calculated and regarded as the estimated variance of the logarithm HR/RR/

Table 1. General study characteristics of cohort studies

Author, year	Country	Outcome	Population; follow-up (years)	Age at entry	Sex	Components of score	Adjustment	Multivariate adjusted	Quality score (max. 9 points)
Ax <i>et al.</i> , 2014	SWE	Prostate cancer	1,044; 13	50	M	1. ↑ legumes/nuts/seeds; 2. ↑ cereals; 3. ↑ fruits; 4. ↑ vegetables/potatoes; 5. ↑ fish; 6. ↑ PUFA:SFA ratio; 7. ↓ meat and meat products; 8. ↓ dairy products	Energy intake, smoking, regular PA and level of education	HR: 1.04 [0.43, 2.49] for third (≥ 6) vs. first tertile (≤ 2)	6
Bamia <i>et al.</i> , 2013 Agnoli <i>et al.</i> , 2013	EPIC ITA	Colorectal cancer	520,000; 8	25–70	M/W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ MUFA:SFA ratio; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ dairy products	Sex, age, BMI, PA, educational level, smoking status at enrolment and energy intake	HR: 0.89 [0.80, 0.99] for third (≥ 6) vs. first tertile (≤ 3)	8
Bosire <i>et al.</i> , 2013	USA	Prostate cancer	293,464; 8.9	50–71	M	1. ↑ legumes; 2. ↑ whole grain products; 3. ↑ fruits; 4. ↑ nuts; 5. ↑ vegetables; 6. ↑ fish; 7. ↑ MUFA:SFA ratio; 8. ↔ alcohol; 9. ↓ red and processed meats	Age, ethnicity, educational level, BMI, smoking, PA, family history of prostate cancer, diabetes and energy	HR: 0.97 [0.91, 1.03] For fifth (≥ 8) vs. first quintile (≤ 2)	8
Buckland <i>et al.</i> , 2013 Trichopoulou <i>et al.</i> , 2010 Cottet <i>et al.</i> , 2009	EPIC GRE FRA	Breast cancer	335,062; 8	35–70	W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ olive oil; 7. ↓ meat and poultry; 8. ↓ dairy products	BMI, height, education level, PA, smoking status, menopausal status, age at menopause, oral contraception use, breastfeeding, age at menarche, age at first full-term pregnancy, use of hormone replacement therapy, SFA intake, alcohol intake and total energy intake	HR: 0.94 [0.83, 1.07] for third (≥ 10 –16) vs. first (≤ 6) tertile	8
Buckland <i>et al.</i> , 2011	EPIC	Cancer mortality	40,622; 13.4	29–69	M/W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ MUFA:SFA ratio; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ dairy products	BMI, waist circumference, education level, PA, smoking status and intensity and total energy intake	HR: 0.92 [0.75, 1.13] for third (≥ 11 –18) vs. first (≤ 6) tertile	8
Buckland <i>et al.</i> , 2010	EPIC	Gastric adenocarcinoma	485,044; 8.9	35–70	M/W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ MUFA:SFA ratio; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ dairy products	Age, sex, BMI, education, smoking habit and total energy	HR: 0.67 [0.47, 0.96] for third (≥ 11 –18) vs. first (≤ 6) tertile	8
Cade <i>et al.</i> , 2011	UK	Breast cancer	33,731; 9	35–69	W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ PUFA:SFA ratio; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ poultry 10. ↓ dairy products	Age, energy intake, menopausal status, calorie-adjusted, BMI, PA, oral contraceptive use, hormone replacement therapy use, smoking status, parity, age at menarche, ethanol, total days breastfeeding, socioeconomic class and level of education	HR: 0.65 [0.42, 1.01] for sixth (≥ 7) vs. first sextile (≤ 2)	7

Table 1. General study characteristics of cohort studies (Continued)

Author, year	Country	Outcome	Population; follow-up (years)	Age at entry	Sex	Components of score	Adjustment	Multivariate adjusted	Quality score (max. 9 points)
Cottet <i>et al.</i> , 2005	FRA	Colorectal cancer	442; 3	35–75	M/W	Factor analysis: ↑ olive oil, fruit, vegetables, legumes, lean meat, fish; ↓ coffee, meat, beer, fats, whole grain bread and vegetables and delicatessen	Age, treatment group and presence of proximal adenomas at inclusion	OR: 0.30 [0.09, 0.98] factor analysis	5
Couto <i>et al.</i> , 2013	SWE	Breast cancer	49,258; 16	30–49	W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ MUFA:SFA ratio; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ dairy products	History of breast cancer in mother and/or sister(s), personal history of benign breast disease, smoking status, BMI, height, age at first birth and number of children, educational level, age at menarche, total energy intake, consumption of beverages, potatoes, sweets and eggs	RR: 1.42 [0.99, 2.03] for fifth (≥8) vs. first quintile (≤2)	8
Couto <i>et al.</i> , 2011 (Benetou excluded)	EPIC	Cancer incidence	335,873; 8.7	25–70	M/W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ MUFA:SFA ratio; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ dairy products	Age, sex, smoking habit, years of education, BMI, height, PA, total energy, consumption of potatoes, eggs, confectionery and nonalcoholic beverages	HR: 0.96 [0.94, 0.98] for two increments in MD score	8
Benetou <i>et al.</i> , 2008	GRE		25,623; 7.3	20–86				HR: 0.78 [0.64, 0.95] for third (≥6) vs. first tertile (≤3)	
Fung <i>et al.</i> , 2006	USA	Breast cancer	71,058; 18	30–55	W	1. ↑ legumes; 2. ↑ whole grain products; 3. ↑ fruits; 4. ↑ nuts; 5. ↑ vegetables; 6. ↑ fish; 7. ↑ MUFA:SFA ratio; 8. ↔ alcohol; 9. ↓ red and processed meats	Age, smoking habit, BMI, multivitamins, energy, PA, family history of breast cancer and of benign breast disease, menopause, HRT, weight change since age 18 years	RR: 0.98 [0.86, 1.11] for fifth (≥8) vs. first quintile (≤2)	8
Fung <i>et al.</i> , 2010	USA	Colorectal cancer	121,700 + 51,529; ≤26	30–55, 40–75	W/M	1. ↑ legumes; 2. ↑ whole grain products; 3. ↑ fruits; 4. ↑ nuts; 5. ↑ vegetables; 6. ↑ fish; 7. ↑ MUFA:SFA ratio; 8. ↔ alcohol; 9. ↓ red and processed meats	Age, BMI, alcohol intake, family history, PA, aspirin use	RR: 0.88 [0.75, 1.04] for fifth (≥8) vs. first quintile (≤2)	9
Kenfield <i>et al.</i> , 2013	USA	Prostate cancer	51,529; 24	40–75	M	1. ↑ legumes; 2. ↑ whole grain products; 3. ↑ fruits; 4. ↑ nuts; 5. ↑ vegetables; 6. ↑ fish; 7. ↑ MUFA:SFA ratio; 8. ↔ alcohol; 9. ↓ red and processed meats	Age in months, time period, energy, BMI, PA, smoking status, and prostate-specific antigen screening history, race, height, history of diabetes, family history of prostate cancer, multivitamin use	HR: 0.95 [0.90, 1.00] for third (≥6) vs. first tertile (≤3)	9

Table 1. General study characteristics of cohort studies (Continued)

Author, year	Country	Outcome	Population; follow-up (years)	Age at entry	Sex	Components of score	Adjustment	Multivariate adjusted	Quality score (max. 9 points)
Knoops <i>et al.</i> , 2004	USA	Cancer mortality	2,339; 10	70–90	M/W	1. ↑ legumes/nuts/seeds; 2. ↑ cereals; 3. ↑ fruits; 4. ↑ vegetables/potatoes; 5. ↑ fish; 6. ↑ MUFA:SFA ratio; 7. ↓ meat and meat products; 8. ↓ dairy products	Age, sex, smoking habit, PA, BMI, dietary habits, alcohol, years of education, study population	HR: 0.90 [0.70, 1.16] for cutoff (≥4) vs. (<4)	8
Lagiou <i>et al.</i> , 2006	SWE	Cancer mortality	42,237; 12	30–49	W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/ nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ MUFA:SFA ratio; 7. ↔ alcohol; 8. ↓ meat and meat products; 9. ↓ dairy products	Age, height, BMI, smoking habit, PA, education, energy, consumption of potatoes, eggs, PUFA, sweets and nonalcoholic beverages	HR: 0.80 [0.57, 1.12] for third (≥6) vs. first tertile (≤3)	8
Li <i>et al.</i> , 2013	USA	Esophageal and gastric cancer	494,968; 11	51–70	M/W	1. ↑ legumes; 2. ↑ whole grain products; 3. ↑ fruits; 4. ↑ nuts; 5. ↑ vegetables; 6. ↑ fish; 7. ↑ MUFA:SFA ratio; 8. ↔ alcohol; 9. ↓ red and processed meats	Age, sex, race, smoking, education, BMI, PA, usual activity, and total energy intake and alcohol intake	HR: 1.10 [0.76, 1.59]; HR: 0.75 [0.52, 1.08]; HR: 0.44 [0.22, 0.88]; HR: 0.91 [0.66, 1.25] for third (≥7) vs. first tertile (≤2)	9
Martinez- Gonzalez <i>et al.</i> , 2012	SPA	Cancer mortality	15,353; 6.8	38	M/W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ MUFA:SFA ratio; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ dairy products	Age, sex, years of university of education, BMI, smoking, PA, hours per day spent watching television, history of depression, baseline hypertension, baseline hypercholesterolemia, total energy intake, egg consumption, potato consumption and adoption of special diets	HR: 1.02 [0.73, 1.44] for third (≥6) vs. first tertile (≤2)	8
Menotti <i>et al.</i> , 2011	ITA	Cancer mortality	1,221; 40	45–64	M	1. ↑ bread, 2. ↑ cereals (pasta); 3. ↑ potatoes; 4. ↑ vegetables, 5. ↑ fish 6 ↑ oil; 7. ↓ milk; 8. ↓ sugar; 9. ↓ fruit; 10. ↓ alcoholic beverages	Age, body weight	HR: 0.84 (0.74, 0.96) factor analysis	5
Mitrou <i>et al.</i> , 2007	USA	Cancer mortality	214,284, 166,012; 10	50–71	M/W	1. ↑ legumes; 2. ↑ whole grain products; 3. ↑ fruits; 4. ↑ nuts; 5. ↑ vegetables; 6. ↑ fish; 7. ↑ MUFA:SFA ratio; 8. ↔ alcohol; 9. ↓ red and processed meats	Age, race, smoking habit, energy, BMI, education and PA	HR: 0.88 [0.78, 0.99]; HR: 0.83 [0.76, 0.91] for third (≥6) vs. first tertile (≤3)	9

Table 1. General study characteristics of cohort studies (Continued)

Author, year	Country	Outcome	Population; follow-up (years)	Age at entry	Sex	Components of score	Adjustment	Multivariate adjusted	Quality score (max. 9 points)
Reedy <i>et al.</i> , 2008	USA	Colorectal cancer	492,382; 5	50–70	M/W	1. ↑ legumes; 2. ↑ whole grain products; 3. ↑ fruits; 4. ↑ nuts; 5. ↑ vegetables; 6. ↑ fish; 7. ↑ MUFA:SFA ratio; 8. ↔ alcohol; 9. ↓ red and processed meats	Age, ethnicity, education, BMI, smoking, PA, menopausal hormone therapy and energy intake	RR: 0.72 [0.63, 0.82]; RR: 0.90 [0.72, 1.11] for fifth (≥8) vs. first quintile (≤2)	8
Tognon <i>et al.</i> , 2012	SWE	Cancer mortality, breast, pancreas, colorectal, gastric, prostate	77,151; 9	30–60	M/W	1. ↑ vegetables and potatoes; 2. ↑ fruit and juices; 3. ↑ whole grain cereals; 4. ↑ fish and fish products; 5. ↑ ratio of MUFA+PUFA to SFA; 6. ↔ alcohol intakes; 7. ↓ meat and meat products; 8. ↓ dairy products	Energy intake, age, obesity, smoking status, education and PA	HR: 0.95 [0.91, 0.99]; HR: 1.12 [0.94, 1.29]; HR: 0.82 [0.72, 0.93]; HR: 0.99 [0.89, 1.10]; HR: 1.14 [0.96, 1.35]; HR: 0.88 [0.74, 1.05] for cutoff (>4) vs. (≤4)	8

Abbreviations: ↑: high intake; ↓: low intake; ↔: moderate intake; BMI: body mass index; CRC: colorectal cancer; EPIC: European Prospective Investigation into Cancer and Nutrition; HR: hazard ratio; HRT: hormone replacement therapy; MUFA: monounsaturated fat; OR: odds ratio; PA: physical activity; PUFA: polyunsaturated fat; RR: risk ratio; SFA: saturated fat.

Table 2. General study characteristics of case-control studies

Author, year	Country	Outcome	Cases/Controls	Age	Sex	Components of score	Adjustment	Multiple adjusted	Quality score (max. 9 points)
Bessaoud <i>et al.</i> , 2012	FRA	Breast cancer	437/922	25–85	W	1. ↑ vegetables; 2. ↑ fruits; 3. ↑ fish; 4. ↑ olive oil	Energy intake, education, parity, breast feeding, age at first full term pregnancy, duration of ovulatory activity, BMI, PA, first degree family history of breast cancer	OR: 0.97 [0.63, 1.49] cluster analysis	6
Bosetti <i>et al.</i> , 2003	ITA	Oral/pharyngeal, esophageal, laryngeal cancer	598/1,491, 304/743, 460/1,088	57 60 61	M/W	1. ↑ MUFA/SFA; 2. ↑ cereals; 3. ↑ legumes; 4. ↑ fruits; 5. ↑ vegetables; 6. ↓ meat; 7. ↓ dairy products; 8. ↔ alcohol	Age, sex, study center, education, smoking, BMI, total energy intake	OR: 0.40 [0.26, 0.62], OR: 0.26 [0.13, 0.52], OR: 0.23 [0.13, 0.41] for fifth (≥6) vs. first quintile (<3)	6
Bosetti <i>et al.</i> , 2013	ITA	Pancreas cancer	688/2,204	18–86	M/W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ olive oil; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ dairy products	Centre, age, sex, year of interview, education, BMI, tobacco smoking, alcohol consumption, history of diabetes and total energy intake	OR: 0.48 [0.35, 0.67] for fifth (≥6) vs. first quintile (<3)	6
Demetriou <i>et al.</i> , 2012	GRE	Breast cancer	935/817	40–70	W	1. olive oil; 2. fruit; 3. vegetables or salad; 4. fruit and vegetables 5. legumes; 6. fish; 7. wine; 8. meat; 9. bread and rice whole-grain bread	Age, family history, HRT use, PA, age at menarche, height and BMI in postmenopausal women	OR: 0.99 [0.70, 1.40] for fourth (≥8) vs. first quartile (≤4)	6
Dixon <i>et al.</i> , 2007	USA	Colorectal cancer	3,592/33,971	55–74	M/W	1. ↑ vegetables; 2. ↑ legumes; 3. ↑ fruit and nuts; 4. ↑ cereals; 5. ↑ fish; 6. ↑ MUFA/SFA ratio; 7. ↔ alcohol intakes; 8. ↓ meat and meat products; 9. ↓ dairy products	Center, age, energy intake, ethnic origin, education, BMI, PA, smoking, aspirin use, ibuprofen use, calcium supplement use and hormone replacement therapy	OR: 0.79 [0.68, 0.92] for sixth (≥6) vs. first sextile (≤2)	8
Kontou <i>et al.</i> , 2012	GRE	Colorectal cancer	250/250	40–75	M/W	1. ↑ non refined cereals; 2. ↑ potatoes; 3. ↑ fruit; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ legumes; 7. ↔ alcohol intakes; 8. ↓ meat and meat products; 9. ↓ dairy products	Age, sex and family history of CRC	OR: 0.88 [0.84, 0.92] per 1/75 units	6
Möller <i>et al.</i> , 2013	SWE	Prostate cancer	1,482/1,108	35–79	M	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ MUFA:SFA ratio; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ dairy products	Age, region of residence, education, smoking status, BMI, energy intake, PA, history of diabetes and family history of prostate cancer	OR: 1.03 [0.81, 1.31] for third (≥6) vs. first tertile (≤3)	7

Table 2. General study characteristics of case-control studies (Continued)

Author, year	Country	Outcome	Cases/Controls	Age	Sex	Components of score	Adjustment	Multiple adjusted	Quality score (max. 9 points)
Murtaugh <i>et al.</i> , 2008	USA	Breast cancer	Hispanic: 757/867; non-Hispanic: 1,524/1,598	25–79	W	Via factor analysis: MD pattern included high factor loadings for liquor consumption, poultry, seafood, vegetables, salad greens and high-fat salad dressings	Age, medical center, education, ethnicity, menopausal status, family history, smoking, PA, energy intake, dietary fiber, dietary calcium, height, parity, BMI, hormone exposure	OR: 0.76 [0.58, 1.00] for fourth vs. first quartile (factor analysis)	7
Nkondjock <i>et al.</i> , 2007	CAN	Breast cancer	89/94	<65	W	1. ↑ vegetables and potatoes; 2. ↑ fruit and juices; 3. ↑ whole grain cereals; 4. ↑ fish and fish products; 5. ↑ MUFA+PUFA:SFA ratio; 6. ↔ alcohol; 7. ↓ meat and meat products; 8. ↓ dairy products	Age, PA and total energy intake	OR: 0.54 [0.17, 1.72] for third (≥6) vs. first tertile (≤3)	6
Praud <i>et al.</i> , 2013	ITA	Gastric cancer	999/2,628	19–80	M/W	1. ↑ legumes; 2. ↑ cereals; 3. ↑ fruits/nuts; 4. ↑ vegetables; 5. ↑ fish; 6. ↑ olive oil; 7. ↔ alcohol; 8. ↓ meat and poultry; 9. ↓ dairy products	Age, sex, study, year of interview, education, BMI, tobacco smoking, family history and total energy intake	OR: 0.57 (0.45, 0.70) for the fourth (≥6) vs. first quartile (≤3)	6
Samoli <i>et al.</i> , 2010	GRE	Larynx, esophagus cancer	239/194	61	M/W	1. ↑ legumes; 2. ↑ whole grain products; 3. ↑ fruits; 4. ↑ nuts; 5. ↑ vegetables; 6. ↑ fish; 7. ↑ MUFA:SFA ratio; 8. ↔ alcohol; 9. ↓ red and processed meats	Age and sex, as well as mutually among the indicated variables	OR: 0.59 [0.39, 0.77] for three units increase	6
Wu <i>et al.</i> , 2009	USA	Breast cancer	1,248/1,148	25–74	W	Via factor analysis: 1. ↑ vegetables; 2. ↑ legumes; 3. ↑ fruit/nuts; 4. ↑ cereals; 5. ↑ fish and seafood; 6. ↑ MUFA:SFA. Ratio; 7. ↓ meat; 8. ↓ dairy products; 9. ↓ carbohydrates; 10. ↔ alcohol	Age, specific ethnicity, education, birthplace, years of residence in the US, PA, marital status, parity, age at menarche, type of menopause, age at menopause, BMI and energy intake	RR: 0.70 [0.48, 1.02] for sixth (≥8) vs. first sextile (≤3)	7

Abbreviations: ↑: high intake; ↓: low intake; ↔: moderate intake; BMI: body mass index; HRT: hormone replacement therapy; MUFA: monounsaturated fat; OR: odds ratio; PA: physical activity; PUFA: polyunsaturated fat; RR: risk ratio; SFA: saturated fat.

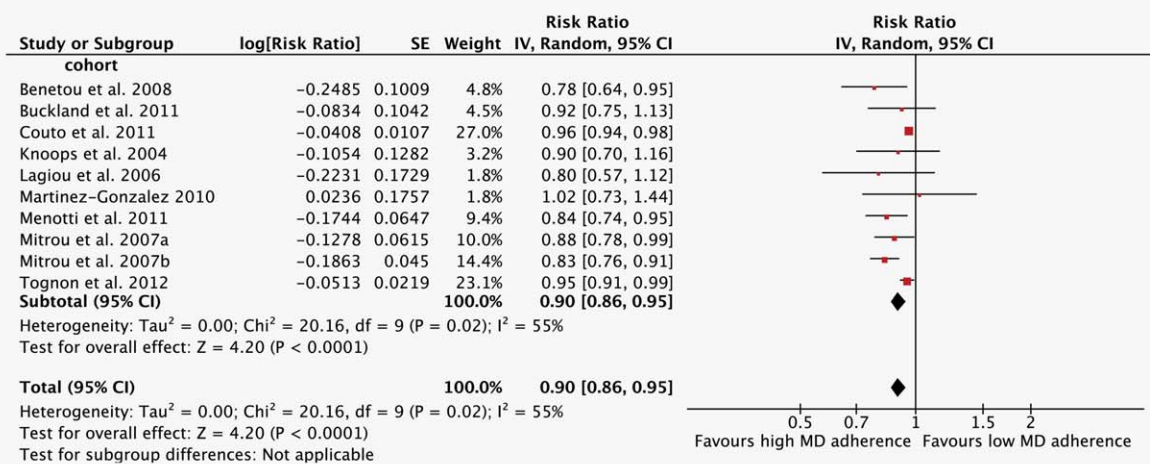


Figure 1. Forest plot showing pooled relative risks (RRs) with 95% CI for overall cancer mortality/incidence risk for nine cohort studies. I^2 : inconsistency; MD: Mediterranean diet; SE: standard error; tau: estimate between study variance. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

OR using an inverse variance method.⁵⁰ Studies were grouped according to the different clinical outcomes (overall risk of cancer mortality/incidence and risk of colorectal cancer/breast cancer/prostate cancer/gastric cancer/aerodigestive cancer/pancreatic cancer). Subgroup analysis was performed for cohort studies and case-control studies. Data were pooled if outcomes were reported by at least two studies. Heterogeneity was estimated by the Cochrane Q test together with the I^2 statistic. An I^2 value >50% indicates substantial heterogeneity across studies.⁵¹ Funnel plots were used to assess potential publication bias. To determine the presence of publication bias, we assessed the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors. In addition, Egger test was performed to test for potential publication bias.⁵² Moreover, to investigate possible sources of heterogeneity across studies, we performed a meta-regression analysis to investigate the effects of various characteristics of studies on the study estimates of RRs. All analyses were conducted using the Review Manager by the Cochrane Collaboration (version 5.2) and STATA 12.0 (Stata-Corp, College Station, TX).

Results

Literature search and study characteristics

A total of 21 cohort studies (25 reports)^{13,14,16–27,31,33,34,37–42,44,45} and 12 case-control^{15,28–30,32,35,36,43,46–49} studies extracted from 6,934 articles met the inclusion criteria and were enclosed for meta-analysis. The detailed steps of the meta-analysis article search (Supporting Information) and selection process are given as a flow chart in Supporting Information Figure 1.

General study characteristics are given in Tables 1 and 2. Sample size varied between 442 and 520,000 (incidence/mortality cases; aerodigestive: 848; breast: 15,912; colorectal: 9,062; gastric: 1,434; pancreas: 47; prostate: 29,867 and types

of cancer not specified: 39,858 cases) with a follow-up time ranging from 3 to 24 years for cohort studies, and a sample size between 183 and 33,563 for case-control studies. The total number of subjects in the included studies was 1,431,461.

Main outcomes

According to the different clinical outcomes, overall risk of cancer mortality/incidence was evaluated in nine cohorts, breast cancer risk in five cohorts and five case-control studies, colorectal cancer risk in five cohorts and two case-control studies, prostate cancer risk in four cohorts and one case-control study, gastric cancer risk in three cohorts and one case-control study, aerodigestive cancer (esophageal/pharyngeal) risk in one cohort and two case-control studies and pancreatic cancer in one cohort and one case-control study.

Using a random effects model, we found that the highest adherence score to an MD was significantly associated with a reduced risk of overall cancer mortality/incidence (relative risk, RR: 0.90, 95% CI 0.86–0.95; $p < 0.0001$, $I^2 = 55\%$) (Fig. 1), colorectal cancer (RR: 0.86, 95% CI 0.80–0.93, $p < 0.0001$, $I^2 = 62\%$; cohort, RR: 0.86, 95% CI 0.76–0.97, $p = 0.02$; case-control studies, RR: 0.85, 95% CI 0.78–0.94, $p = 0.001$) (Fig. 2), breast cancer (RR: 0.95, 95% CI 0.84–1.06, $p = 0.35$, $I^2 = 52\%$; cohort, RR: 1.01, 95% CI 0.88–1.16, $p = 0.89$; case-control studies, RR: 0.82, 95% CI 0.69–0.97, $p = 0.02$) (Fig. 3), prostate cancer (RR: 0.96, 95% CI 0.92–0.99, $p = 0.03$, $I^2 = 0\%$; cohort, RR: 0.95, 95% CI 0.92–0.99, $p = 0.02$; case-control studies, RR: 1.03, 95% CI 0.81–1.31, $p = 0.81$) (Fig. 4), gastric cancer (RR: 0.82, 95% CI 0.59–1.13, $p = 0.29$, $I^2 = 85\%$; cohort, RR: 0.91, 95% CI 0.69–1.20, $p = 0.51$; case-control, RR: 0.57, 95% CI 0.45–0.72, $p < 0.00001$) (Supporting Information Fig. 2), aerodigestive cancer (RR: 0.44, 95% CI 0.26–0.77, $p = 0.003$, $I^2 = 83\%$; cohort, RR: 0.68, 95% CI 0.34–1.36, $p = 0.27$; case-control studies, RR: 0.34, 95% CI

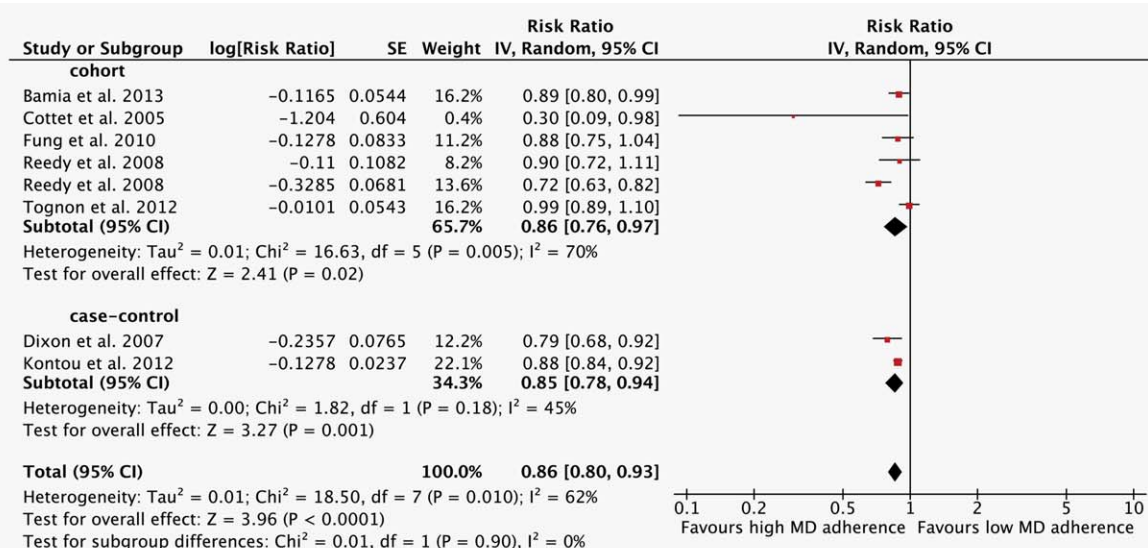


Figure 2. Forest plot showing pooled relative risks (RRs) with 95% CI for risk of colorectal cancer for five cohort and two case-control studies. I^2 : inconsistency; MD: Mediterranean diet; SE: standard error; tau: estimate between study variance. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

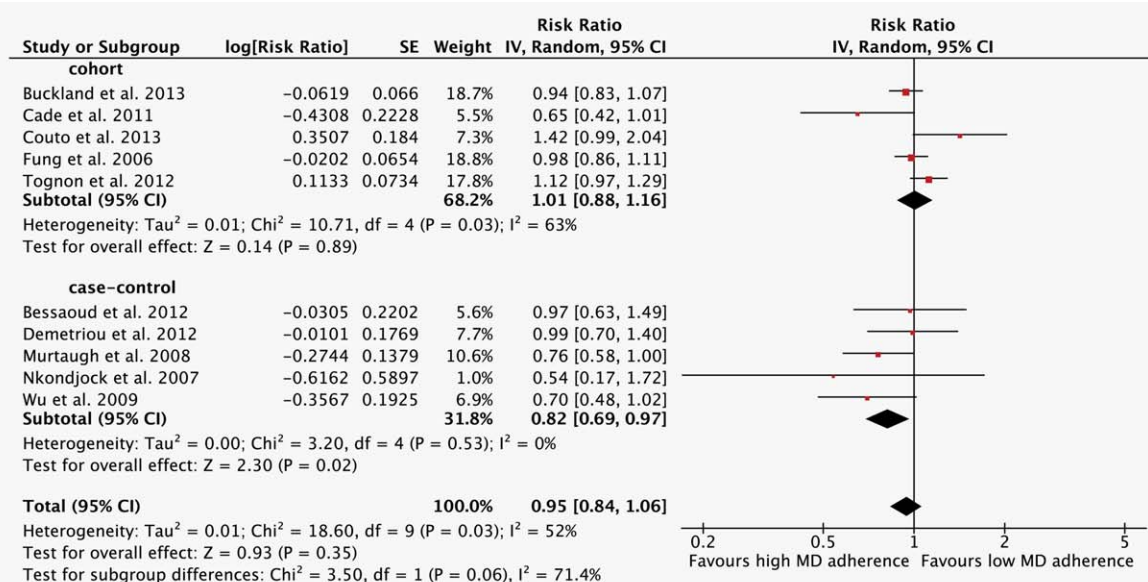


Figure 3. Forest plot showing pooled relative risks (RRs) with 95% CI for risk of breast cancer for five cohort and five case-control studies. I^2 : inconsistency; MD: Mediterranean diet; SE: standard error; tau: estimate between study variance. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

0.18–0.77, $p = 0.001$) (Supporting Information Fig. 3) and pancreatic cancer (RR: 0.64, 95% CI 0.38–1.08, $p = 0.09$, $I^2 = 89\%$) (Supporting Information Fig. 4).

Sensitivity analyses

Sensitivity analysis was performed for breast cancer comparing premenopausal vs. postmenopausal women. Results were not significantly different from the ones obtained in the primary analysis. Furthermore, sensitivity analyses were performed for outcomes presented by least five studies (no

merging of cohort and case-control studies was done in the sensitivity analysis) taking into account country of origin, follow-up time and quality of studies. Comparing studies performed in Europe- vs. US-based investigations confirmed the results of the main analysis, with the exception for colorectal cancer yielding no significant effect for EU countries studies. In addition, studies with a follow-up length ≥ 8 years resulted in similar results when compared to short-term studies (< 8 years follow-up). Similarly, high-quality studies could confirm the results of the main analysis.

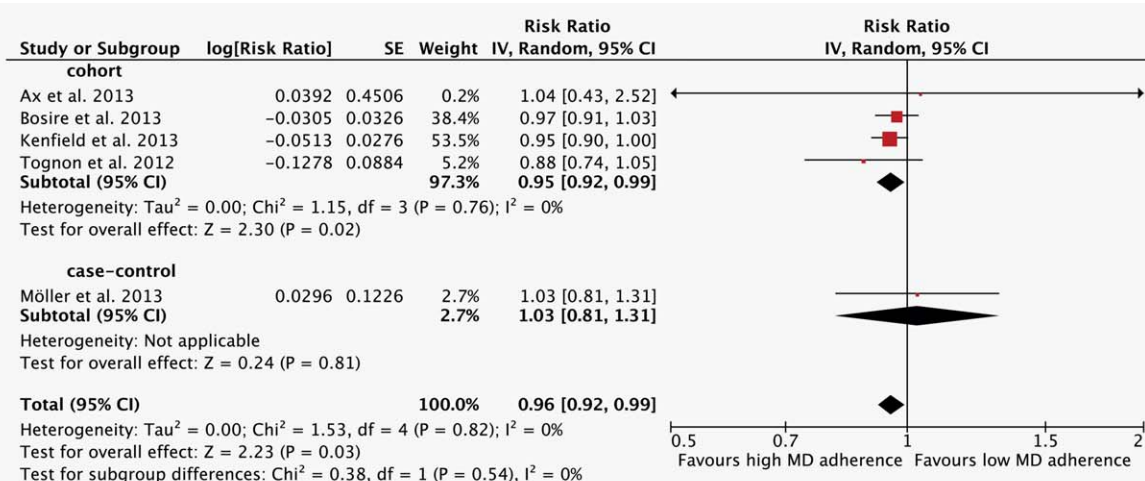


Figure 4. Forest plot showing pooled relative risks (RRs) with 95% CI for risk of prostate cancer for four cohort and one case-control study. I^2 : inconsistency; MD: Mediterranean diet; SE: standard error; tau: estimate between study variance. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Publication bias

The Egger's linear regression tests provided evidence for a potential publication bias for overall cancer mortality/risk ($p = 0.025$) following comparison of the highest vs. lowest adherence to MD category. However, including only studies on cancer mortality provides no evidence of publication bias. No evidence of publication bias could be detected for risk of breast, colorectal, gastric and prostate cancer as well. In contrast, there was some evidence for publication bias regarding risk of aerodigestive cancer, which is probably due to the fact that only three studies in total were investigating these types of cancer ($p = 0.040$). Funnel plots were generated only when more than ten studies were available for a comparison. The funnel plots for risk of overall cancer mortality/incidence as well as risk of breast and colorectal cancer indicate moderate asymmetry, suggesting that publication bias cannot be completely excluded as a factor of influence on the present meta-analysis. It remains possible that small studies with inconclusive results have not been published or failed to do so.

Meta-regression

To investigate the effects of various study characteristics on the study estimates of the RRs, we conducted a meta-regression analysis (only for cohort studies, because discrepancies compared to case-control are too excessive) by grouping studies according to specific characteristics, *i.e.*, sample size, age of the patients and length of follow-up. There was a trend for a borderline significant association between risk of colorectal cancer and age ($p = 0.052$).

Discussion

The main findings of this meta-analysis suggest that the highest adherence to MD category was associated with a significant reduction of overall risk of cancer mortality/incidence by 10%. These findings are in accordance with pre-

vious meta-analyses analyzing the effects of adherence to MD on overall cancer risk.^{9,10} However, no previous meta-analyses have synthesized the effects of MD on different cancer types. The risk of colorectal cancer could be reduced by 14%, and the risk of prostate cancer by 4%. No significant risk reduction could be observed for breast, gastric and pancreatic cancer. However, a subgroup analysis pooling case-control studies showed that the risk of breast cancer could be reduced by 18% when adhering to an MD. The association between adherence to MD and risk of aerodigestive cancer provides evidence for risk reduction from case-control studies, which could not be confirmed by pooling the cohort studies regarded to provide a higher level of evidence. According to the World Cancer Research Fund grading system, the present systematic review provides convincing evidence that high adherence to MD is associated with a reduced risk of overall cancer mortality/incidence as well as colorectal cancer, probable evidence that high adherence to MD is associated with a reduced risk of prostate cancer and probable evidence that high adherence to MD has no effects on breast and gastric cancer risk.⁵³ Randomized controlled trials are the main source of evidence to establish causality; however, until to date, only one randomized controlled trial investigating this topic has been published. The results of Lyon Diet Heart Study including 605 subjects showed a 61% reduction of cancer risk in the MD study arm compared with the control group.⁵⁴ With respect to the largest MD trial, the PREDIMED study including 7,447 subjects, no data on cancer incidence have yet been published.⁵⁵

Cancer incidence might be influenced by dietary factors through several mechanism, *e.g.*, suppression of spontaneous mutations, affecting cell proliferation and the methylation of DNA and induction of apoptosis.⁵⁶ The main component of the MD is olive oil, consumed in high amount by MD populations. A recent systematic review and meta-analysis of 19

observational studies suggested a 0.66 times lower odds of having any type of cancer when comparing the highest category of olive oil consumption with the lowest figure.⁵⁷

MD is characterized by a high content of protective food groups and clearly supports the fact that not total fat but the type of fat plays a significant role in cancer progression. In an article published in 2008, Sotiroudis and Kyrtopoulos stated that the phenolic content of extra-virgin and virgin olive oil is able to specifically affect cancer-regulated oncogenes.⁵⁸ Apart from being a stabilizer of oleic acid protecting the unsaturated fat against oxidants, phenolic compounds present in extra virgin olive oil may exert strong chemopreventive effects *via* a variety of distinct mechanism as well, including both direct antioxidant effects and actions on cancer cell signaling and cell cycle progression.⁶ In this context, one should keep in mind that owing to processing losses, in refined olive oils only a small proportion of the native phenolic ingredients remain.⁷

Other main components of an MD such as vegetables, fruits, whole grain foods and fish are related to reduced risk of colorectal, esophageal and breast cancer, respectively.^{59–62} Dietary fiber may decrease the risk of colorectal cancer through a variety of mechanism (e.g., an enlargement of the bulk of stool thereby reducing transit time). Furthermore, bacterial fermentation of fiber to short-chain fatty acids in the gut may promote colonic cell differentiation and normal cell apoptosis.⁶³ The lower risk for cancer seen with high consumption of fruits and vegetables may be explained through multiple biological effects of flavonoids, including antioxidant activity, inhibition of inflammation, antimutagenic and antiproliferative properties as well as involvement in cell signaling, cell cycle regulation and angiogenesis.⁶⁴ The MD score includes low consumption of red meat and dairy products. Regarding red and processed meat, a recent meta-analysis of 21 observational studies showed that high consumption is associated with increased risk of colorectal cancer.⁶⁵ However, data of meta-analysis of cohort studies yielded inconsistent results for dairy products and cancer risk.^{66,67} A possible link between adherence to an MD and reduced risk of prostate cancer is formed by the high consumption of tomatoes. Experimental data have shown that lycopene, the most relevant functional component in tomatoes, has beneficial in cancer prevention. Lycopene exerts antioxidant capacities and is involved in downregulation mechanisms of the inflammatory response.^{68,69} The protective effect of the MD on cancer risk may be mediated through several biological mechanisms such as chronic inflammation and oxidative stress.

Another important point that should be noted is the recommendation of moderate alcohol consumption in the MD score. According to the World Cancer Research Fund

(WCRF) 2007 report, alcoholic beverages providing ≥ 30 g of ethanol/day are associated with increased risks of different cancers (e.g., pharynx, larynx, esophagus, colorectal, breast and liver).⁵³ However, moderate alcohol consumption in an MD is characterized by a threshold of <20 g of ethanol/day with a focus on red wine containing secondary plant substances such as polyphenols associated with reduced risk from fatal and nonfatal vascular events.

Several limitations of this systematic review should be considered. Studying the relationship between diet and cancer is complex and requires adjusting and stratifying by multiple confounding variables and careful interpretation. The MD is not a homogenous pattern, and heterogeneity on the score items exists. However, the bulk of the included studies used the well-established MD score by Trichopoulou *et al.*⁵ Furthermore, the design of cohort studies has various limitations compared with randomized controlled trials. Cohort studies have caveats, including a reliance on nutritional assessment methods with validity and reliability being lower compared to randomized controlled trials. Compared to cohort studies, case-control studies are still prone to error, possible measurement error and recall bias should be taken into account. A further limitation exists in the different adjustment for potential confounders. This difference could have determined a residual confounding within the studies. Using MD patterns in American and Nordic populations might not adequately represent conformity with the traditional MD components that are also characteristics of a general prudent dietary pattern. In addition, three of the included case-control studies reported no adjustment for energy intake.

The present systematic review has several strengths as well. Many of the studies, especially cohort studies, were of high methodological quality. Overall more than 1.4 million subjects were included in the meta-analysis, thus providing the power to detect statistically significant RR as well to assess publication bias.

In conclusion, the present systematic review and meta-analysis shows that high adherence to MD is associated with reduced risk from cancer mortality/incidence, colorectal and prostate cancer, and is therefore of clinical as well as public health significance. Over the past 40 years, dietary habits have changed enormously in Europe compared with the traditional MD pattern of the 1960s in southern Europe. Countries in the south of Europe have tended to move toward the dietary pattern typical of northern countries by increasing the consumption of dietary fat, especially fat from animal origin.⁷⁰ Because of these data, recommendations to reverse this trend might be beneficial with respect to primary prevention of distinct types of cancer by dietary measures.

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Diet Quality as Assessed by the Healthy Eating Index, the Alternate Healthy Eating Index, the Dietary Approaches to Stop Hypertension Score, and Health Outcomes: A Systematic Review and Meta-Analysis of Cohort Studies

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ABSTRACT

Dietary patterns consider synergistic effects compared with isolated foods or nutrients on health outcomes. The aim of this systematic review and meta-analysis was to examine the associations of diet quality as assessed by the Healthy Eating Index (HEI), the Alternate Healthy Eating Index (AHEI), and the Dietary Approaches to Stop Hypertension (DASH) score and the risk of all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, type 2 diabetes mellitus, and neurodegenerative diseases. A literature search was performed using the electronic databases MEDLINE, SCOPUS, and EMBASE with an end date of May 10, 2014. Study-specific risk ratios were pooled using a random effect model by the Cochrane software package Review Manager 5.2. Fifteen cohort studies (34 reports), including 1,020,642 subjects, met the criteria and were included in the meta-analysis. Diets of the highest quality, as assessed by the HEI, AHEI, and DASH score, resulted in a significant risk reduction (RR) for all-cause mortality (RR 0.78, 95% CI 0.76 to 0.80; $P < 0.00001$; $I^2 = 61\%$, 95% CI 20% to 81%), cardiovascular disease (incidence or mortality) (RR 0.78, 95% CI 0.75 to 0.81; $P < 0.00001$; $I^2 = 45\%$, 95% CI 13% to 66%), cancer (incidence or mortality) (RR 0.85, 95% CI 0.82 to 0.88; $P < 0.00001$; $I^2 = 77\%$, 95% CI 68% to 84%), and type 2 diabetes mellitus (RR 0.78, 95% CI 0.72 to 0.85; $P < 0.00001$; $I^2 = 74\%$, 95% CI 52% to 86%). Differences observed for neurodegenerative diseases were not significant. Egger regression tests provided no evidence of publication bias. Diets that score highly on the HEI, AHEI, and DASH are associated with a significant reduction in the risk of all-cause mortality, cardiovascular disease, cancer, and type 2 diabetes mellitus by 22%, 22%, 15%, and 22%, respectively, and therefore is of high public health relevance.

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NUTRIENTS ARE NOT CONSUMED IN ISOLATION, and it is the synergy of nutrients within the context of a healthy dietary pattern that is frequently associated with a reduced risk of disease, such as cardiovascular disease (CVD) or cancer, and all-cause mortality.¹⁻³ However, the question of the preventive potential of specific dietary patterns with respect to CVD and cancer outcomes is still unclear.^{4,5} In this context, it might be of interest to investigate not only specific nutrients but also the effectiveness of dietary patterns that can be assessed using corresponding indexes. In a systematic literature review, Barbaresko and colleagues⁶ reported that healthy diets were associated with a reduction in inflammatory biomarkers; however, they could not find any prospective studies to confirm these correlations. In addition, a systematic review of cohort studies revealed that diets that scored high on diet quality indexes such as the Healthy Eating Index (HEI)-2005

and the Alternate Healthy Eating Index (AHEI) were associated with a protective effect against colorectal cancer.⁷ In the United States the HEI, the AHEI, and the Dietary Approaches to Stop Hypertension (DASH) score are distinct diet quality indexes of major public health importance.

The HEI was developed to measure how well diets conform to federal dietary guidance. The original 1995 version had 10 components that measured the five major food groups, four nutrients that should be consumed in moderation, and variety.⁸ The HEI was revised in 2008 to reflect the 2005 Dietary Guidelines for Americans, and scores were based on amounts of foods and nutrients consumed per 1,000 kcal energy intake rather than on absolute amounts.⁹ The HEI was updated and validated to reflect the 2010 Dietary Guidelines for Americans.^{10,11}

The AHEI, based on features of the original HEI, was developed by McCullough and colleagues¹² in 2002. Food

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choices and macronutrients predictive of chronic disease risk were a focus.^{12,13} The AHEI used an absolute intake approach in contrast to a nutrient density basis.¹² The most recent version of the AHEI-2010 was developed by Chiuve and colleagues¹³ in 2012.

In addition to the HEI and AHEI, a third diet quality index is the DASH score, but there are several versions of the DASH score, and no single score is considered to optimally reflect the DASH diet.¹⁴⁻¹⁷ To date, the DASH score established by Fung and colleagues¹⁶ is the most frequently used in the literature and is therefore described here. This DASH score is based on eight components: high intake of fruits, vegetables, nuts and legumes, low-fat dairy products, and whole grains, as well as low intake of sodium, sweetened beverages, and red and processed meats.¹⁶ Clinical trials have demonstrated that the DASH dietary pattern was associated with substantially lower blood pressure, one of the leading risk factors for CVD.¹⁸

The aim of this systematic review and meta-analysis was to examine cohort studies that investigated the association of diet quality as assessed by the HEI, AHEI, and DASH score and health status (ie, risk of all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, type 2 diabetes mellitus, and neurodegenerative diseases).

METHODS

The review protocol has been registered in PROSPERO: The International Prospective Register of Systematic Reviews (crd.york.ac.uk/prospero/index.asp Identifier: CRD42013006561).

Data Sources and Searches

A literature search was performed using the electronic databases MEDLINE, EMBASE, and SCOPUS (until May 10, 2014) with no restrictions to calendar date, and language using the following search terms: *Healthy Eating Index* and *DASH diet*. For an example of a full search strategy, see Figure 1 (available online at www.andjrn.org). Moreover, the reference lists from retrieved articles were checked to search for further relevant studies. This systematic review was planned, conducted, and reported in adherence to standards of quality for reporting meta-analyses of observational studies in epidemiology guidelines.¹⁹ The literature search was conducted independently by both authors, with disagreements resolved by consensus.

Study Selection

Studies were included in the meta-analysis if they met all of the following criteria: a cohort study; evaluated the association of diet quality as assessed by the HEI, and/or AHEI, and/or DASH on all-cause mortality, and/or cardiovascular mortality or incidence, and/or cancer mortality or incidence, and/or risk of type 2 diabetes mellitus, and/or neurodegenerative disease; presented relative risks (RRs) and/or hazard ratios (HRs) with corresponding 95% CIs or the data necessary to calculate these; study participants were free of outcome(s) of interest at the onset of the study; outcomes of interest include all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, risk of type 2 diabetes mellitus, and neurodegenerative disease; and when a study seemed to have been published in duplicate, the version

containing the most comprehensive information was selected.

Data Extraction and Quality Assessment

The following data were extracted from each study: the first author's last name, year of publication, study origin, cohort name, outcome parameter, sample size, study length (follow-up in years), age at entry, sex, diet quality score, adjustment factors, study quality score, and risk estimates (HR or RR or highest vs lowest category) with their corresponding 95% CIs. If separate risk estimates for male and female participants were available in one study, data were treated as two separate studies only in the case that the data for the whole population were missing. When a study provided several risk estimates, the multivariate adjusted model was chosen. To assess the study quality, a 9-point scoring system according to the Newcastle-Ottawa Quality Assessment Scale was used (see Figure 2 [available online at www.andjrn.org]). Hence, the full score was nine, and a high-quality study in the present analysis was defined by a threshold of ≥ 7 points.²⁰ Data extraction and quality assessment were performed by one author (L.S.).

HEI, AHEI, and DASH Components and Scoring

HEI. The HEI has 10 components (grains, vegetables, fruits, milk, meat, total fat, saturated fat, cholesterol, sodium, and variety)⁸ and minimum and maximum scores between 0 and 10 points (overall scoring range=0 to 100).

HEI-2005. The HEI-2005 has 12 components: total fruit (5 points); whole fruit (5 points); total vegetables (5 points); dark green and orange vegetables and legumes (5 points); total grains (5 points); whole grains (5 points); milk (10 points); meat and beans (10 points); oils (10 points); saturated fat (10 points); sodium (10 points); and calories from solid fats, alcoholic beverages (ie, beer, wine, and distilled spirits) and added sugars (20 points) (overall scoring range=0 to 100).⁹

HEI-2010. Twelve components: total fruit (5 points); whole fruit (5 points); total vegetables (5 points); greens and beans (5 points); whole grains (10 points); dairy (10 points); total protein foods (5 points); seafood and plant proteins (5 points); fatty acids (polyunsaturated fatty acid+monounsaturated fatty acid-to-saturated fatty acid ratio) (10 points); refined grains (10 points); sodium (10 points); and empty calories from solid fats, alcoholic beverages (ie, beer, wine, and distilled spirits) and added sugars (20 points) (overall scoring range=0 to 100).¹⁰

AHEI. Nine components (vegetables, fruit, nuts and soy protein, ratio of white to red meat, cereal fiber, *trans* fat, polyunsaturated-to-saturated fat ratio, duration of multivitamin use, and alcohol). With the exception of multivitamins, minimum and maximum scores for each component ranged from 0 to 10. For alcohol, the criteria for the minimum score was either 0 servings/day (ie, below moderate alcohol consumption) or >3.5 servings/day for men and >2.5 servings/day for women; criteria for the maximum score was 1.5 to 2.5 servings/day for men, and 0.5 to 1.5 servings/day for women. For duration of multivitamin use, the minimum score was 2.5

and the maximum score was 7.5. Therefore, the overall scoring range was 2.5 to 87.5.¹²

AHEI-2010. Eleven components: vegetables, fruit, whole grains, sugar-sweetened beverages and fruit juice, nuts and legumes, red/processed meat, *trans* fat, long-chain (n-3) fatty acids (eicosapentaenoic acid and docosahexaenoic acid), polyunsaturated fat, sodium, and alcohol (overall scoring range=0 to 110).¹³

DASH. Two versions have eight components (fruit, vegetables, nuts and legumes, whole grains, low-fat dairy, sodium, red and processed meats, and sweetened beverages), but they are scored differently. Either minimum and maximum component scores between 1 and 5,¹⁶ minimum and maximum component scores between 0 and 10,¹⁵ The third version has 11 components with minimum and maximum component scores 0 to 1.¹⁴ Therefore, overall scoring range was either 8 to 40,¹⁶ 0 to 80,¹⁵ or 0 to 11.¹⁴

Statistical Analysis

Random effects meta-analyses methods were used to combine evidence of comparisons between the lowest and highest quintiles of HEI, AHEI, and DASH scores among various outcomes.²¹ Because outcomes were not very rare and heterogeneity modeling was deemed important, the random effects model was used. To evaluate the weight of each study, the standard error for the logarithm HR or RR of each study was calculated and regarded as the estimated variance of the logarithm HR or RR, using an inverse variance method. Studies were grouped according to the different clinical outcomes (ie, all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, type 2 diabetes, and neurodegenerative diseases). Subgroup analyses were performed for the HEI, AHEI, and for the DASH score. Furthermore, subgroup analyses were performed for the HEI (comparing the original HEI vs HEI-2005 vs HEI-2010) and the AHEI (comparing the original AHEI vs AHEI-2010). Data were pooled if outcomes were reported by at least two studies. Heterogeneity was estimated by the Cochrane Q test together with the I^2 statistic. An I^2 value >50% indicates substantial heterogeneity across studies.²² The *heterogi* command in Stata was used to calculate the CIs for the heterogeneity estimates. Funnel plots were used to assess potential publication bias. The presence of publication bias was assessed by the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors. In addition, Egger test was performed to test for potential publication bias.²³ All analyses were conducted using Review Manager (version 5.2, 2012, Cochrane Collaboration) and Stata version 13.0 (2013, StataCorp). Sensitivity analyses were performed assuming statistical heterogeneity ($I^2 \geq 50\%$) with the *metaan* command in Stata.²⁴

RESULTS

Literature Search and Study Characteristics

The detailed steps of the article selection process are given as a flow chart in Figure 3.

Altogether, 15 cohort studies (34 reports) extracted from 6,506 articles met the inclusion criteria and were included in the meta-analysis.^{12-16,25-53}

General study characteristics are given in Table 1. Sample size varied between 862 and 537,218 with a follow-up time ranging from 5 to ≥ 24 years. The total number of subjects in the included studies was 1,020,642.

Main Outcomes

According to the different clinical outcomes, all-cause mortality was evaluated in four cohort studies,^{25,32,44,53} cardiovascular mortality or incidence in 12 cohort studies (17 reports),^{12-14,16,25-27,31,32,36,43,44,46-48,52,53} cancer mortality or incidence in six cohort studies (17 reports) (breast, esophageal, gastric, pancreatic, prostate cancer, hepatocellular carcinoma, and head and neck cancer in one cohort study, respectively, and colorectal cancer in two cohort studies),^{2,13,25-28,30,32-34,39-41,44,49,51,53} risk of type 2 diabetes mellitus in six cohort studies (7 reports),^{13,15,29,35,42,45,50} and risk of neurodegenerative disease in two cohort studies.^{37,38}

Using a random effects model, we found that the highest association of diet quality as assessed by the HEI, AHEI, or DASH score was significantly associated with a reduced risk of all-cause mortality (RR 0.78, 95% CI 0.76 to 0.80; $P < 0.00001$; $I^2 = 61\%$, 95% CI 20% to 81%) (Figure 4), cardiovascular mortality or incidence (RR 0.78, 95% CI 0.75 to 0.81; $P < 0.00001$; $I^2 = 45\%$, 95% CI 13% to 66%) (Figure 5), cancer mortality or incidence (RR 0.85, 95% CI 0.82 to 0.88; $P < 0.00001$; $I^2 = 77\%$, 95% CI 68% to 84%) (Figure 6), cancer types (Figure 7), and risk of type 2 diabetes (RR 0.78, 95% CI 0.72 to 0.85; $P < 0.00001$; $I^2 = 74\%$, 95% CI 52% to 86%) (Figure 8). No significant association could be observed for Parkinson's disease (RR 0.90, 95% CI 0.54 to 1.51; $P = 0.69$; $I^2 = 39\%$, 95% CI 0% to 81%) (Figure 9). The corresponding enumerative data are summarized in Table 2.

Subgroup analysis showed an association between diets that scored highly on the HEI, AHEI, and DASH and reduced risk of colorectal, esophageal, pancreatic, and prostate cancer.

Subgroup analysis suggested that all diets that scored highly on the included dietary indexes (HEI, AHEI, and DASH score) were associated with a reduced risk of CVD. However, the subgroup analysis for risk of cancer indicated that diets that scored highly on the original versions of the HEI and AHEI were not significantly associated with reduced cancer risk, whereas diets that scored highly on the more recent diet quality indexes (ie, HEI-2005, HEI-2010, AHEI-2010, and DASH) were significantly associated with reduced cancer risk (data not shown).

Sensitivity Analyses

Sensitivity analysis was performed including only US-cohort studies because the HEI, AHEI, and DASH score diet quality indexes were established in the United States. Results were not significantly different from the ones obtained in the primary analysis (Table 3, available online at www.adnjrnl.org). However, including only US-based studies for risk of type 2 diabetes reduced heterogeneity. Furthermore, sensitivity analyses were performed taking follow-up time and quality of studies into account. Including studies with a follow-up length ≥ 8 years resulted in similar results compared with the main analysis (Table 4, available online at www.andjrnl.org). Likewise, high-quality studies confirmed the results of the primary analysis (Table 5, available online at www.andjrnl.org). Because participants from the same

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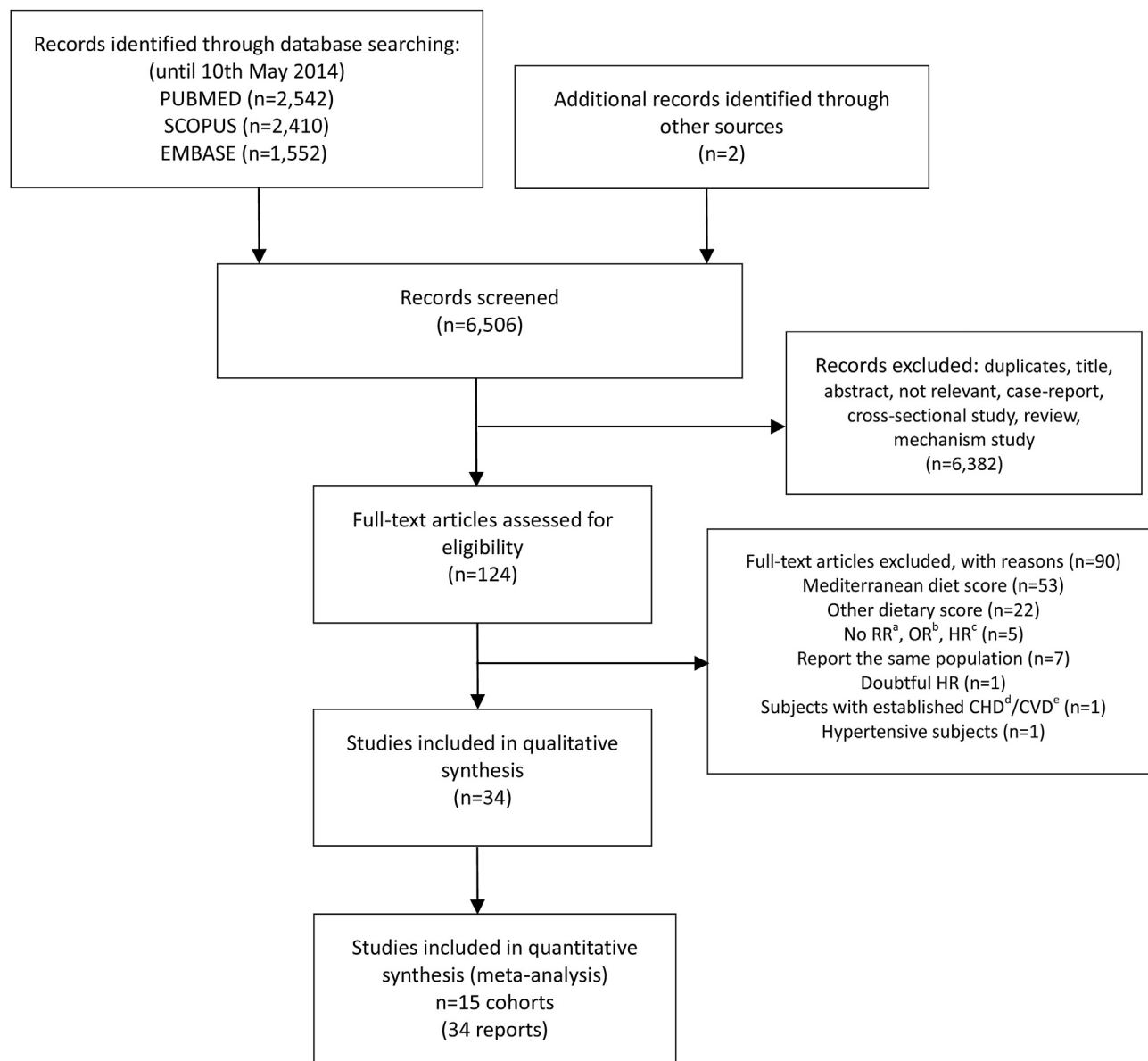


Figure 3. Flow chart for the article selection process for a meta-analysis of the associations of diet quality as assessed by the Healthy Eating Index, Alternate Healthy Eating Index, and Dietary Approaches to Stop Hypertension score and health outcomes. ^aRR=relative risk. ^bOR=odds ratio. ^cHR=Hazard ratio. ^dCHD=congenital heart disease; ^eCVD=cardiovascular disease.

cohorts were included in several analyses, a sensitivity analysis for risk of CVD, cancer, and type 2 diabetes was performed including only the cohorts referring to the most recent score. Again, the results of the primary analysis were confirmed (Table 6, available online at www.andjrn.org). Meta-analyzing the sex effect yielded the following RR for CVD: women 0.77 (95% CI 0.75 to 0.80) vs men 0.79 (95% CI 0.73 to 0.85); for cancer: women 0.86 (95% CI 0.81 to 0.92) vs men 0.84 (95% CI 0.79 to 0.90); and for type 2 diabetes: women 0.75 (95% CI 0.70 to 0.80) vs men 0.76 (95% CI 0.69 to 0.84), respectively (Tables 7 and 8, available online at www.andjrn.org). Because for CVD and cancer mortality and incidence rates were combined, we performed sensitivity analysis comparing mortality vs incidence (CVD incidence RR 0.77

[95% CI 0.74 to 0.80], cancer incidence RR 0.86 [95% CI 0.82 to 0.91], CVD mortality RR 0.79 [95% CI 0.76 to 0.84], and cancer mortality RR 0.82 [95% CI 0.79 to 0.85]). We observed no significant differences (data not shown).

Considering that heterogeneity is often underestimated or not identified, we repeated the analyses assuming high heterogeneity ($I^2 \geq 75\%$) to assess the validity of our results under this assumption. Results from these sensitivity and main analyses generally agreed and the conclusions drawn were identical.

Publication Bias

Egger linear regression tests (performed with at least 10 studies) provided no evidence for a potential publication bias

Table 1. Summary of characteristics and results of 15 cohort studies (34 reports) that investigate the associations of diet quality as assessed by the Healthy Eating Index (HEI), the alternate Healthy Eating Index (AHEI), and the Dietary Approaches to Stop Hypertension (DASH) score and the risk of all-cause mortality, cardiovascular mortality, cardiovascular incidence, cancer mortality, cancer incidence, type 2 diabetes mellitus, and neurodegenerative diseases

Author, y	Country	Cohort	Outcome	Population (n) Follow-up (y)	Age at entry (y)	Sex	Diet Quality Index	Adjustment	RR ^a /HR ^b (95% CI) Multivariate adjusted ^c	Ottawa Quality Assessment Scale ²⁰ (max 9)
Agnoli and colleagues, 2011 ⁵²	Europe	EPIC ^d	Stroke events	40,681 7.9	35-74	F ^e /M ^f	HEI (2005) DASH score	Sex, smoking status, education, nonalcoholic energy intake, and BMI ^g	HEI (2005) HR 0.89 (0.60-1.32) DASH score HR 0.75 (0.51-1.10)	8
Akbaraly and colleagues, 2011 ³²	Great Britain	Whitehall II	All-cause mortality CVD ^h mortality Cancer mortality	7,319 18	39-63	F/M	AHEI	Sex, age, ethnic group, marital status, occupational grade, smoking habits, physical activity, total energy intake, BMI categories, inflammatory markers, metabolic syndrome, prevalence of CVD, dyslipidemia, hypertension, and type 2 diabetes	HR 0.76 (0.61-0.95) HR 0.58 (0.37-0.91) HR 0.80 (0.58-1.11)	9
Arem and colleagues, 2013 ³⁴	United States	NIH-AARP ⁱ	Pancreatic cancer incidence	537,218 10.5	50-71	F/M	HEI (2005)	Daily caloric intake, sex, diabetes, BMI, and smoking status (categories)	HR 0.90 (0.85-0.95)	9
Belin and colleagues, 2011 ³¹	United States	WHI ^j observational study	observational CVD events	93,676 10	50-79	F	AHEI	Age, race, education, physical activity, BMI, smoking, diabetes medications, taking pills for hypertension ever, and ever taking pills for cholesterol	HR 0.77 (0.70-0.84)	8
Bertoia and colleagues, 2013 ⁴⁸	United States	WHI observational study	Sudden cardiac death	93,122 10.5	50-79	F	DASH score	Age, energy, race, income, smoking status, physical activity, pulse in 60 sec, waist- to-hip ratio, BMI, coronary artery disease, heart failure, diabetes, and hypertension	HR 0.86 (0.54-1.38)	8
Bosire and colleagues, 2013 ³³	United States	NIH-AARP	Prostate cancer incidence	293,464 8.9	50-71	M	HEI (2005) AHEI (2010)	Age, ethnicity, educational level, BMI, smoking, physical activity, family history of prostate cancer, diabetes, and energy	HEI (2005) HR 0.92 (0.86-0.98) AHEI (2010) HR 0.93 (0.88-0.99)	8

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Table 1. Summary of characteristics and results of 15 cohort studies (34 reports) that investigate the associations of diet quality as assessed by the Healthy Eating Index (HEI), the alternate Healthy Eating Index (AHEI), and the Dietary Approaches to Stop Hypertension (DASH) score and the risk of all-cause mortality, cardiovascular mortality, cardiovascular incidence, cancer mortality, cancer incidence, type 2 diabetes mellitus, and neurodegenerative diseases (*continued*)

Author, y	Country	Cohort	Outcome	Population (n) Follow-up (y)	Age at entry (y)	Sex	Diet Quality Index	Adjustment	RR ^a /HR ^b (95% CI) Multivariate adjusted ^c	Ottawa Quality Assessment Scale ²⁰ (max 9)
Chiuve and colleagues, 2012 ¹³	United States	HP ^k and NHS ^l	CVD events Cancer incidence Type 2 diabetes	71,495 41,029 ≥24	30-55 40-75	F/M	HEI (2005) AHEI (2010)	Age, energy, smoking status, BMI, aspirin, physical activity, vitamin E supplementation, family history of myocardial infarction, family history of colon cancer; in the analysis in women, models adjusted for family history of breast cancer, menopausal status, and use of hormone therapy; history of hypertension and history of hypercholesterolemia, except where cancer was the only outcome.	HEI (2005) HR 0.79 (0.71-0.88) HR 0.90 (0.84-0.96) HR 0.82 (0.76-0.89) AHEI (2010) HR 0.76 (0.71-0.81) HR 0.94 (0.89-0.98) HR 0.67 (0.61-0.74)	8
de Koning and colleagues, 2011 ⁴²	United States	HP	Type 2 diabetes	41,615 ≤20 y	ND ^m	M	HEI (2005) AHEI DASH score	Smoking, physical activity, coffee intake, family history of type 2 diabetes, BMI, and total energy	HEI (2005) HR 0.96 (0.84-1.10) AHEI HR 0.77 (0.67-0.88) DASH score HR 0.75 (0.65-0.87)	8
Fitzgerald and colleagues, 2012 ³⁶	United States	WHS ⁿ	CVD incidence	39,876 14.6	≥45	F	DASH score	Randomization status, age, smoking, time-varying postmenopausal status, time-varying hormone therapy use, alcohol intake, energy intake, physical activity, cigarettes per day, highest education level, BMI, history of high cholesterol, history of hypertension, and family history of congenital heart disease	HR 0.87 (0.70-1.09)	8
Folsom and colleagues, 2007 ¹⁴	United States	Iowa WHS	CVD mortality	20,993 16	55-69	F	DASH score	Age, energy intake, education, BMI, waist/ hip, smoking status, estrogen use, alcohol intake, physical activity, and multivitamin use	HR 0.93 (0.76-1.12)	7

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Table 1. Summary of characteristics and results of 15 cohort studies (34 reports) that investigate the associations of diet quality as assessed by the Healthy Eating Index (HEI), the alternate Healthy Eating Index (AHEI), and the Dietary Approaches to Stop Hypertension (DASH) score and the risk of all-cause mortality, cardiovascular mortality, cardiovascular incidence, cancer mortality, cancer incidence, type 2 diabetes mellitus, and neurodegenerative diseases (*continued*)

Author, y	Country	Cohort	Outcome	Population (n) Follow-up (y)	Age at entry (y)	Sex	Diet Quality Index	Adjustment	RR ^a /HR ^b (95% CI) Multivariate adjusted ^c	Ottawa Quality Assessment Scale ²⁰ (max 9)
Fung and colleagues, 2006 ³⁰	United States	NHS	Breast cancer incidence	71,058 18	30-55	F	HEI AHEI	Age, BMI, physical activity, age at menopause and postmenopausal hormone therapy use, smoking, energy intake, family history, weight change since age 18 y, multivitamin use (except for AHEI because it is a component), history of benign breast disease, weight at age 18 y, alcohol (except for AHEI alcohol was part of the scoring criteria).	HEI HR 1.04 (0.92-1.18) AHEI HR 0.99 (0.88-1.11)	8
Fung and colleagues, 2007 ³⁵	United States	NHS	T2D	80,029 18	38-63	F	AHEI	Age, energy intake, smoking, BMI, waist-to- hip ratio, physical activity, family history, menopausal status, and postmenopausal hormone use	RR 0.76 (0.66, 0.88)	8
Fung and colleagues, 2008 ¹⁶	United States	NHS	Congenital heart disease incidence Stroke incidence	88,517 24	34-59	F	DASH score	Age, smoking, BMI, menopausal status, and postmenopausal hormone use, energy intake, multivitamin intake, alcohol intake, family history, physical activity, aspirin use, n-3 fatty acids, and <i>trans</i> fat	RR 0.73 (0.64-0.84) RR 0.83 (0.71-0.96)	8
Fung and colleagues 2010 ⁴¹	United States	HP NHS	Colorectal cancer incidence	M: 87,256 F: 45,490 ≤26	30-55 40-75	F/M	DASH score	Age, BMI, alcohol intake, family history, physical activity, aspirin use, colonoscopy, history of polyps, multivitamin use, pack-years of smoking, and energy intake	RR 0.80 (0.70-0.91)	8
Gao and colleagues 2007 ³⁸	United States	HP NHS	Parkinson's disease	M: 81,676 F: 49,692 16	30-55 40-75	F/M	AHEI	Age, smoking status, use of nonsteroidal anti-inflammatory drugs, and intake of total energy, and caffeine	RR 0.70 (0.51-0.94)	8

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Table 1. Summary of characteristics and results of 15 cohort studies (34 reports) that investigate the associations of diet quality as assessed by the Healthy Eating Index (HEI), the alternate Healthy Eating Index (AHEI), and the Dietary Approaches to Stop Hypertension (DASH) score and the risk of all-cause mortality, cardiovascular mortality, cardiovascular incidence, cancer mortality, cancer incidence, type 2 diabetes mellitus, and neurodegenerative diseases (*continued*)

Author, y	Country	Cohort	Outcome	Population (n) Follow-up (y)	Age at entry (y)	Sex	Diet Quality Index	Adjustment	RR ^a /HR ^b (95% CI) Multivariate adjusted ^c	Ottawa Quality Assessment Scale ²⁰ (max 9)
Kappeler and colleagues, 2013 ⁴⁴	United States	NHANES III ^o	All-cause mortality CVD mortality Cancer mortality	17,611 22	≥18	F/M	HEI	Age, race, sex, cigarette smoking, alcohol consumption, physical activity, socioeconomic status, BMI, marital status, history of hypertension, diabetes, hypercholesterolemia, use of aspirin and ibuprofen, use of mineral and vitamin supplements, family history of diabetes or hypercholesterolemia, hormone replacement therapy, and oral contraceptive use (in women)	HR 0.77 (0.63-0.94) HR 0.85 (0.65-1.11) HR 0.75 (0.51-1.11)	6
Interact, ⁴⁵ 2014	Europe	EPIC	Type 2 diabetes	16,154 11.7	25-70	F/M	AHEI DASH score	Age, study center, sex, physical activity, smoking status, education, total energy intake, BMI, waist circumference	AHEI HR 0.96 (0.86-1.07) DASH score HR 0.95 (0.84-1.07)	8
Levitan and colleagues, 2009 ⁴³	Sweden	Swedish mammography cohort	Heart failure incidence	36,019 7	48-83	F	DASH score	Age, physical activity, energy intake, education status, family history of myocardial infarction at age younger than 60 y, cigarette smoking, living alone, postmenopausal hormone use, self-reported history of hypertension and high cholesterol concentration, BMI, and incident myocardial infarction	RR 0.63 (0.48-0.81)	8
Levitan and colleagues, 2009 ⁴⁶	Sweden	Cohort of Swedish men	Heart failure incidence	38,987 9	45-79	M	DASH score	Age, physical activity, energy intake, education, family history of myocardial infarction at <60 y, cigarette smoking, marital status, self-reported history of hypertension and high cholesterol, BMI, and incident myocardial infarction	HR 0.78 (0.65-0.95)	8

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Table 1. Summary of characteristics and results of 15 cohort studies (34 reports) that investigate the associations of diet quality as assessed by the Healthy Eating Index (HEI), the alternate Healthy Eating Index (AHEI), and the Dietary Approaches to Stop Hypertension (DASH) score and the risk of all-cause mortality, cardiovascular mortality, cardiovascular incidence, cancer mortality, cancer incidence, type 2 diabetes mellitus, and neurodegenerative diseases (*continued*)

Author, y	Country	Cohort	Outcome	Population (n) Follow-up (y)	Age at entry (y)	Sex	Diet Quality Index	Adjustment	RR ^a /HR ^b (95% CI) Multivariate adjusted ^c	Ottawa Quality Assessment Scale ²⁰ (max 9)
Li and colleagues, 2013 ³⁹	United States	NIH-AARP	Esophageal (squamous cell carcinoma, esophageal adenoma carcinoma) and gastric cancer (cardia and noncardia adenoma carcinoma) incidence	494,968 11	51-70	M/F	HEI (2005)	Age, sex, race, smoking, education, BMI, physical activity, usual activity, total energy intake, and alcohol intake	Squamous cell carcinoma HR 0.51 (0.31-0.86) Esophageal adenoma carcinoma HR 0.75 (0.57-0.98) Cardia adenoma carcinoma HR 0.92 (0.67-1.27) Noncardia adenoma carcinoma HR 0.88 (0.65-1.20)	9
Li and colleagues, 2014 ⁴⁹	United States	NIH-AARP	Head and neck cancer incidence	494,967 11	51-70	M/F	HEI (2005)	Age, race, smoking, alcohol intake, education, BMI, physical activity, diabetes, usual activity, and total energy intake	RR M: 0.74 (0.61-0.89) RR F: 0.48 (0.33-0.70)	9
Li and colleagues, 2014 ⁵¹	United States	NIH-AARP	Hepatocellular carcinoma incidence	494,942 11	50-71	M/F	HEI (2010)	Age, race, smoking, alcohol intake, education, BMI, physical activity, diabetes, usual activity, and total energy intake	HR 0.72 (0.53-0.97)	9
Liese and colleagues, 2009 ¹⁵	United States	IRAS ^P	T2D	862 5	ND	M/F	DASH score	Age, sex, race/ethnicity/clinic, glucose tolerance status, family history of diabetes, education, smoking status, energy intake and energy expenditure, BMI, and baseline insulin sensitivity and secretion	Odds ratio 0.64 (0.37-1.13)	4
Lin and colleagues, 2013 ⁴⁷	Taiwan	CVDFACTS ^Q	Stroke incidence	1,420 12	45.5	F/M	DASH score	Sex, baseline age, urinary sodium/creatinine, smoking status, drinking status, physical activity, BMI, blood pressure change, and hypertension medication	HR 0.63 (0.41-0.98)	5

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Table 1. Summary of characteristics and results of 15 cohort studies (34 reports) that investigate the associations of diet quality as assessed by the Healthy Eating Index (HEI), the alternate Healthy Eating Index (AHEI), and the Dietary Approaches to Stop Hypertension (DASH) score and the risk of all-cause mortality, cardiovascular mortality, cardiovascular incidence, cancer mortality, cancer incidence, type 2 diabetes mellitus, and neurodegenerative diseases (*continued*)

Author, y	Country	Cohort	Outcome	Population (n) Follow-up (y)	Age at entry (y)	Sex	Diet Quality Index	Adjustment	RR ^a /HR ^b (95% CI) Multivariate adjusted ^c	Ottawa Quality Assessment Scale ²⁰ (max 9)
McCullough and colleagues, 2000 ²⁷	United States	NHS	CVD incidence Cancer incidence	67,272 12	30-55	F	HEI	Age, smoking, time period, BMI, alcohol intake, physical activity, history of hypertension or hypercholesterolemia, total energy intake, postmenopausal status, postmenopausal hormone use, and multivitamin and vitamin E supplement use	RR 0.86 (0.72-1.03) RR 1.02 (0.93-1.12)	8
McCullough and colleagues, 2000 ²⁶	United States	HP	CVD incidence Cancer incidence	51,529 8	40-75	M	HEI	Age, BMI, smoking, alcohol intake, physical activity, total energy intake, time period, multivitamin use, vitamin E use, and diagnosis of hypercholesterolemia and hypertension at baseline	RR 0.72 (0.60-0.88) RR 1.12 (0.95-1.31)	8
McCullough and colleagues, 2002 ¹²	United States	HP and NHS	CVD events Cancer incidence	M: 38,615 F: 67,271 8-12	30-55 40-75	F/M	AHEI	Age, smoking, time period, BMI, alcohol intake, physical activity, history of hypertension or hypercholesterolemia, total energy intake, postmenopausal status, and postmenopausal hormone use, and vitamin E supplement use	RR F: 0.72 (0.60-0.86) RR F: 0.97 (0.88-1.06) RR M: 0.61 (0.49-0.75) RR M: 1.03 (0.87-1.22)	8
Miller and colleagues, 2013 ²⁸	United States	NIH-AARP	Colorectal cancer incidence	491,841 11	50-71	F/M	DASH score	Age, education, BMI, alcohol intake, smoking, total energy intake, ethnicity, physical activity, and use of hormone replacement therapy in women	RR F: 0.84 (0.73-0.96) RR M: 0.75 (0.68-0.83)	8
Mursu and colleagues, 2013 ²⁵	United States	Iowa WHS	All-cause mortality CVD mortality Cancer mortality	41,836 20	55-69	F	AHEI (2010)	Age, energy intake, marital status, education, place of residence, high blood pressure, BMI, waist-to-hip ratio, hormone replacement therapy, physical activity, and smoking	HR 0.82 (0.77-0.87) HR 0.79 (0.72-0.88) HR 0.88 (0.79-0.98)	7
Qiao and colleagues, 2014 ⁵⁰	United States	WHI	Type 2 diabetes	154,493 7.6	50-79	F	AHEI	Age, education, cigarette smoking, BMI, waist/hip ratio, physical activity, energy intake, family history of diabetes, study arms and hormone therapy use	HR 0.76 (0.70-0.82)	8

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Table 1. Summary of characteristics and results of 15 cohort studies (34 reports) that investigate the associations of diet quality as assessed by the Healthy Eating Index (HEI), the alternate Healthy Eating Index (AHEI), and the Dietary Approaches to Stop Hypertension (DASH) score and the risk of all-cause mortality, cardiovascular mortality, cardiovascular incidence, cancer mortality, cancer incidence, type 2 diabetes mellitus, and neurodegenerative diseases (*continued*)

Author, y	Country	Cohort	Outcome	Population (n) Follow-up (y)	Age at entry (y)	Sex	Diet Quality Index	Adjustment	RR ^a /HR ^b (95% CI) Multivariate adjusted ^c	Ottawa Quality Assessment Scale ²⁰ (max 9)
Reedy and colleagues, 2008 ⁴⁰	United States	NIH-AARP	Colorectal cancer incidence	492,382 5	50-70	F/M	HEI (2005) AHEI	Age, ethnicity, education, BMI, smoking, physical activity, and energy (and menopausal hormone therapy in women only)	HEI (2005) RR F: 0.80 (0.64-0.98) RR M: 0.72 (0.62-0.83) AHEI RR F: 0.83 (0.66-1.05) RR M: 0.71 (0.61-0.82)	8
Reedy and colleagues 2014 ⁵³	United States	NIH-AARP	All-cause mortality CVD mortality Cancer mortality	492,823 15	50-70	F/M	HEI (2010) AHEI (2010) DASH score	Age, race/ethnicity, education, marital status, physical activity, smoking, energy intake, BMI, diabetes, and alcohol	HEI (2010) HR F: 0.77 (0.74-0.80) HR M: 0.78 (0.76-0.80) HR F: 0.79 (0.73-0.85) HR M: 0.85 (0.80-0.89) HR F: 0.82 (0.77-0.87) HR M: 0.76 (0.72-0.80) AHEI (2010) HR F: 0.76 (0.74-0.79) HR M: 0.76 (0.74-0.78) HR F: 0.72 (0.67-0.78) HR M: 0.74 (0.70-0.78) HR F: 0.88 (0.83-0.94) HR M: 0.82 (0.78-0.86) DASH score HR F: 0.78 (0.75-0.81) HR M: 0.83 (0.80-0.85) HR F: 0.78 (0.72-0.83) HR M: 0.86 (0.81-0.91) HR F: 0.82 (0.77-0.88) HR M: 0.80 (0.76-0.84)	9

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Table 1. Summary of characteristics and results of 15 cohort studies (34 reports) that investigate the associations of diet quality as assessed by the Healthy Eating Index (HEI), the alternate Healthy Eating Index (AHEI), and the Dietary Approaches to Stop Hypertension (DASH) score and the risk of all-cause mortality, cardiovascular mortality, cardiovascular incidence, cancer mortality, cancer incidence, type 2 diabetes mellitus, and neurodegenerative diseases (*continued*)

Author, y	Country	Cohort	Outcome	Population (n) Follow-up (y)	Age at entry (y)	Sex	Diet Quality Index	Adjustment	RR ^a /HR ^b (95% CI) Multivariate adjusted ^c	Ottawa Quality Assessment Scale ²⁰ (max 9)
Sääksjärvi and colleagues, 2013 ³⁷	Finland	FMCS ^f	Parkinson's disease	4,524 15	40-79	F/M	AHEI (no alcohol and multivitamin data available)	Age, sex, marital status, community density, geographical area, smoking, BMI, leisure-time physical activity, energy, hypertension, serum total cholesterol, diabetes, and parity in women	RR F: 0.97 (0.38-2.48) RR M: 1.83 (0.65-5.18)	6
Tobias and colleagues, 2012 ²⁹	United States	NHS II	Type 2 diabetes	4413 14	24-44	F	AHEI DASH score	Age, total calorie intake, age at first birth, parity, ethnicity, parental diabetes, oral contraceptive use, menopause, and smoking status	AHEI HR 0.65 (0.46-0.92) DASH score HR 0.68 (0.49-0.94)	6

^aRR=risk ratio.

^bHR=Hazard ratio.

^cIn case of multiple HR/RR values, the order in which data are given correspond to the respective order of outcomes listed in the "Outcome" column.

^dEPIC=European Prospective Investigation into Cancer and Nutrition.

^eF=female.

^fM=male.

^gBMI=body mass index.

^hCVD=cardiovascular disease.

ⁱNIH-AARP=National Institutes of Health-AARP.

^jWHI=Women's Health Initiative observational study.

^kHP=Health Professional Study.

^lNHS=Nurses' Health Study.

^mND=no data available.

ⁿWHS=Women's Health Study.

^oNHANES III=National Health and Nutrition Examination Survey III.

^pIRAS=Insulin Resistance Atherosclerosis Study.

^qCVDFACTS=Cardiovascular Disease Risk Factor Two Township Study.

^rFMCS=Finnish Mobile Clinic Survey.

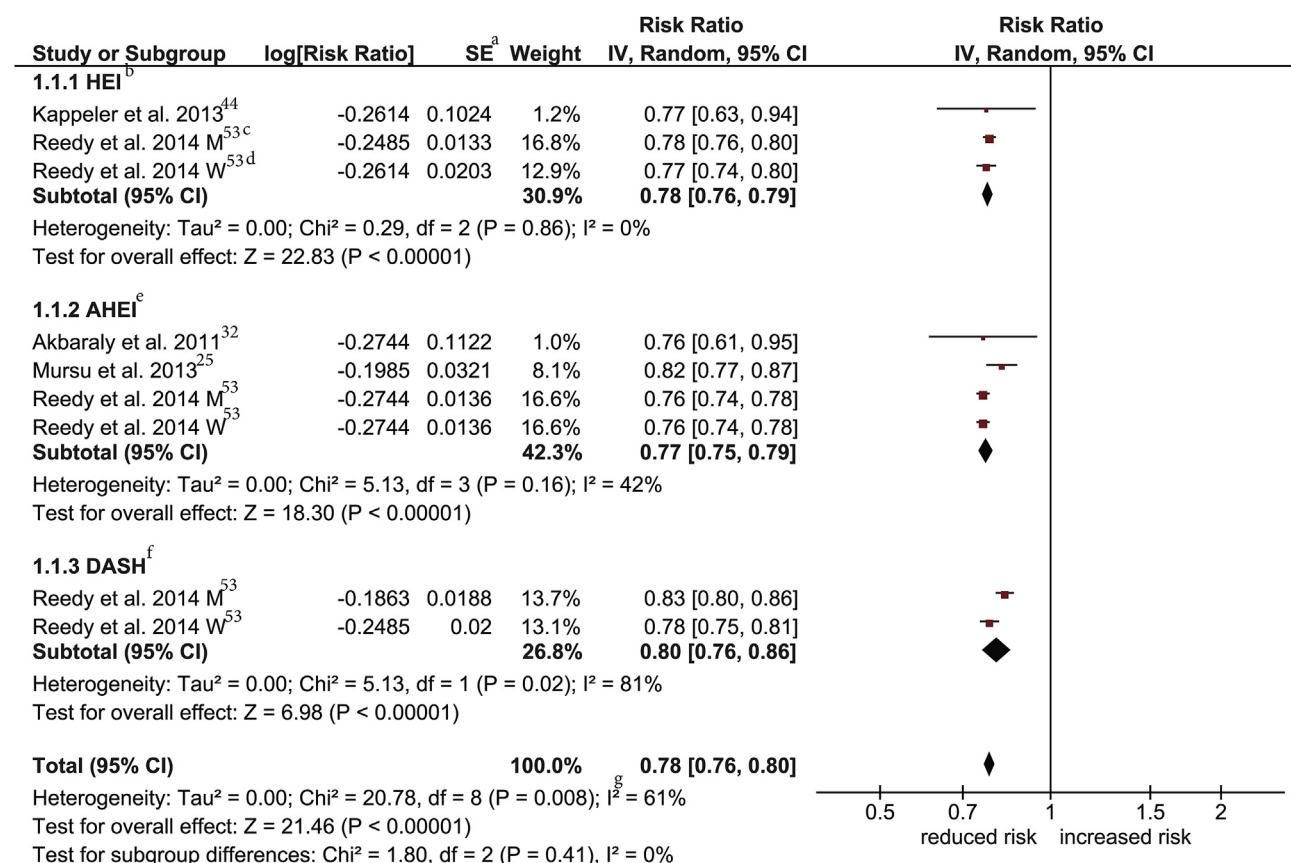


Figure 4. Forest plot showing pooled relative risks (RRs) with 95% CI for the highest diet quality (Healthy Eating Index [HEI], Alternate Health Eating Index [AHEI], and Dietary Approaches to Stop Hypertension [DASH]) vs lowest diet quality category for all-cause mortality. ^aSE=standard error. ^bIncludes the original version, HEI-2005, and HEI-2010. ^cMen. ^dWomen. ^eIncludes the original version, and AHEI-2010. ^fDASH score. ^gI²=inconsistency.

for risk of cardiovascular disease ($P=0.45$), overall cancer mortality or incidence risk ($P=0.35$), and risk of type 2 diabetes ($P=0.61$) following comparison of the highest vs lowest quantiles of HEI, AHEI, and DASH scores (data not shown). Funnel plots were only generated when >10 studies were available for a comparison. The funnel plots for risk of cardiovascular mortality or incidence, cancer mortality or incidence, as well as risk of type 2 diabetes indicate slight asymmetry, suggesting that publication bias cannot be completely excluded as a factor of influence on our meta-analysis (Figures 10, 11, and 12, available online at www.andjrn.org). It is possible that small studies with inconclusive results have not been published.

DISCUSSION

There is an urgent need for interventions to reduce the incidence of CVD, cancer, type 2 diabetes mellitus, and neurodegenerative diseases, which continue to be the most common causes of death in the Western world since 1970.^{54,55} In recent years, dietary pattern analysis has been used increasingly as an alternative method to traditional single nutrient analysis.⁵⁶ The use of dietary patterns avoids focusing on single foods or nutrients that can be correlated with, or interact with, each other and assesses combinations

of food that alone may have effects too small to be identified. Therefore, analysis of food consumption patterns has become a common tool for studying the association between diet and health.^{1,3}

To the best of our knowledge, this is the first systematic review investigating the pooled estimates from studies assessing diet quality using the HEI and AHEI on all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, risk of type 2 diabetes, and risk of neurodegenerative disease, except a systematic review by the US Department of Agriculture.⁵⁷ Furthermore a recent meta-analysis⁵⁸ showed that a DASH-like diet significantly reduces the risk CVD. The main findings of our meta-analysis suggest that diets of the highest quality, as assessed by the HEI, AHEI, and DASH score, were associated with a significant reduction of all-cause mortality risk by 22%, risk of CVD by 22%, risk of cancer by 15%, and risk of type 2 diabetes by 22%, respectively.

As an alternative method to the conventional single nutrient analysis, assessment of dietary patterns is being increasingly applied because it can assess the cumulative effect of the overall diet. There are several statistical limitations with the single-nutrient approaches (intercorrelations); nevertheless, it is relevant to understand the role of individual dietary constituents.⁵⁹ Considering multidimensional aspects of food and non-nutrient components such as

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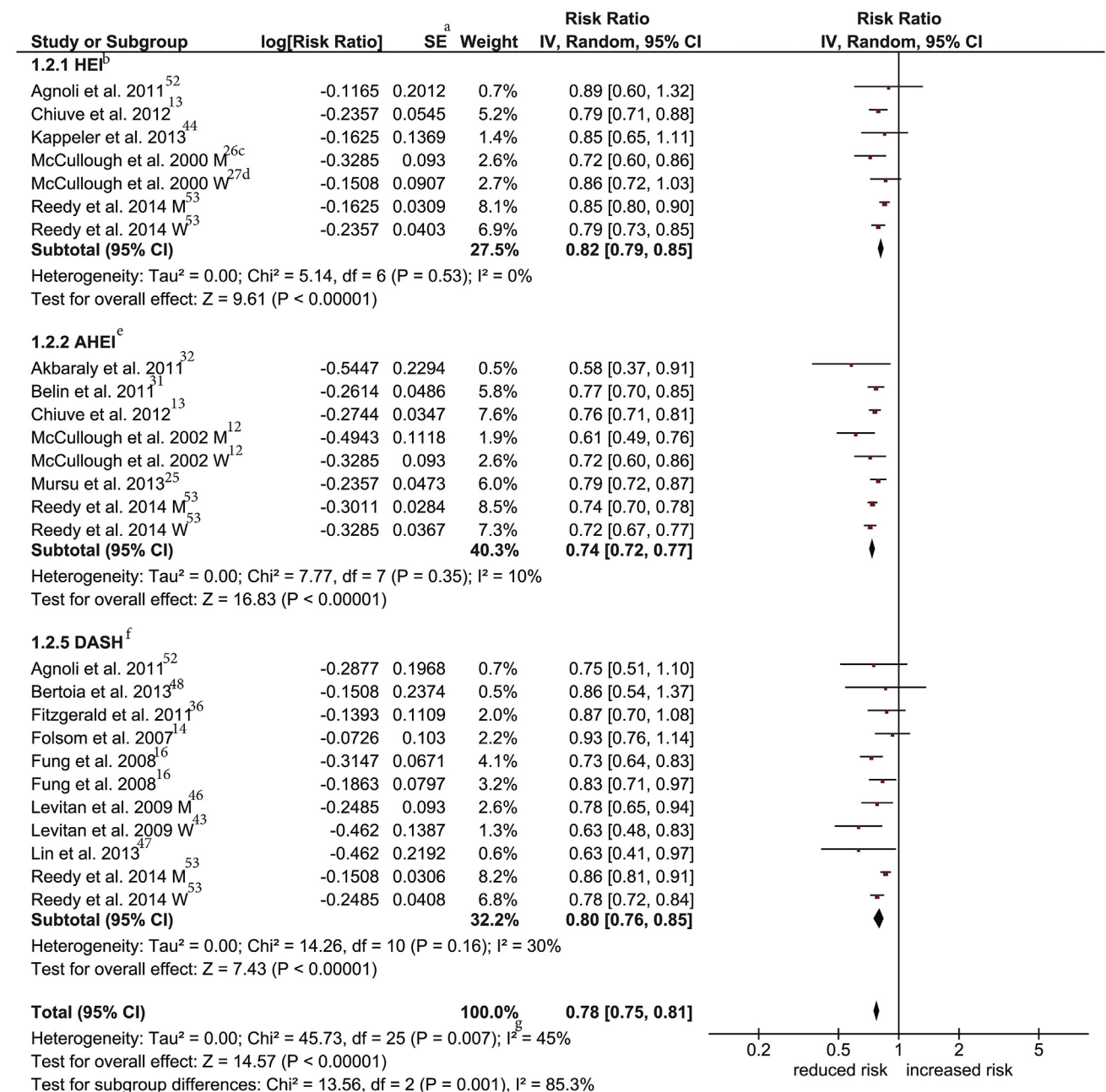


Figure 5. Forest plot showing pooled relative risks (RRs) with 95% CI for the highest diet quality (Healthy Eating Index [HEI], Alternate Health Eating Index [AHEI], and Dietary Approaches to Stop Hypertension [DASH]) vs lowest diet quality category for cardiovascular mortality or incidence. ^aSE=standard error. ^bIncludes the original version, HEI-2005, and HEI-2010. ^cMen. ^dWomen. ^eIncludes the original version and AHEI-2010. ^fDASH score. ^gI²=inconsistency.

biologically active dietary constituents, rather than reducing dietary intake to single nutrients, is a strength of dietary quality indexes.⁶⁰

Variations in results yielded for the dietary quality indexes might be due to the fact that the HEI did not evaluate consumption of red and processed meat in its score. Main components assessed by the HEI, AHEI, and DASH score diet quality indexes are vegetables, fruits, nuts and legumes, and low sodium content. Evidence from epidemiologic studies and clinical trials showed that high consumption of vegetables

and fruits were inversely associated with CVD incidence and mortality.⁶¹ The lower risk for all-cause mortality, CVD, cancer, and diabetes seen with high consumption of fruits and vegetables may be explained through multiple biological effects of magnesium, fiber, potassium, and flavonoids, including antioxidant activity, inhibition of inflammation, and angiogenesis.⁶² Red meat consumption is associated with increased risk of esophageal and colorectal cancer.^{63,64}

The blood pressure-lowering effect of the DASH diet has been shown in randomized controlled trials, and is

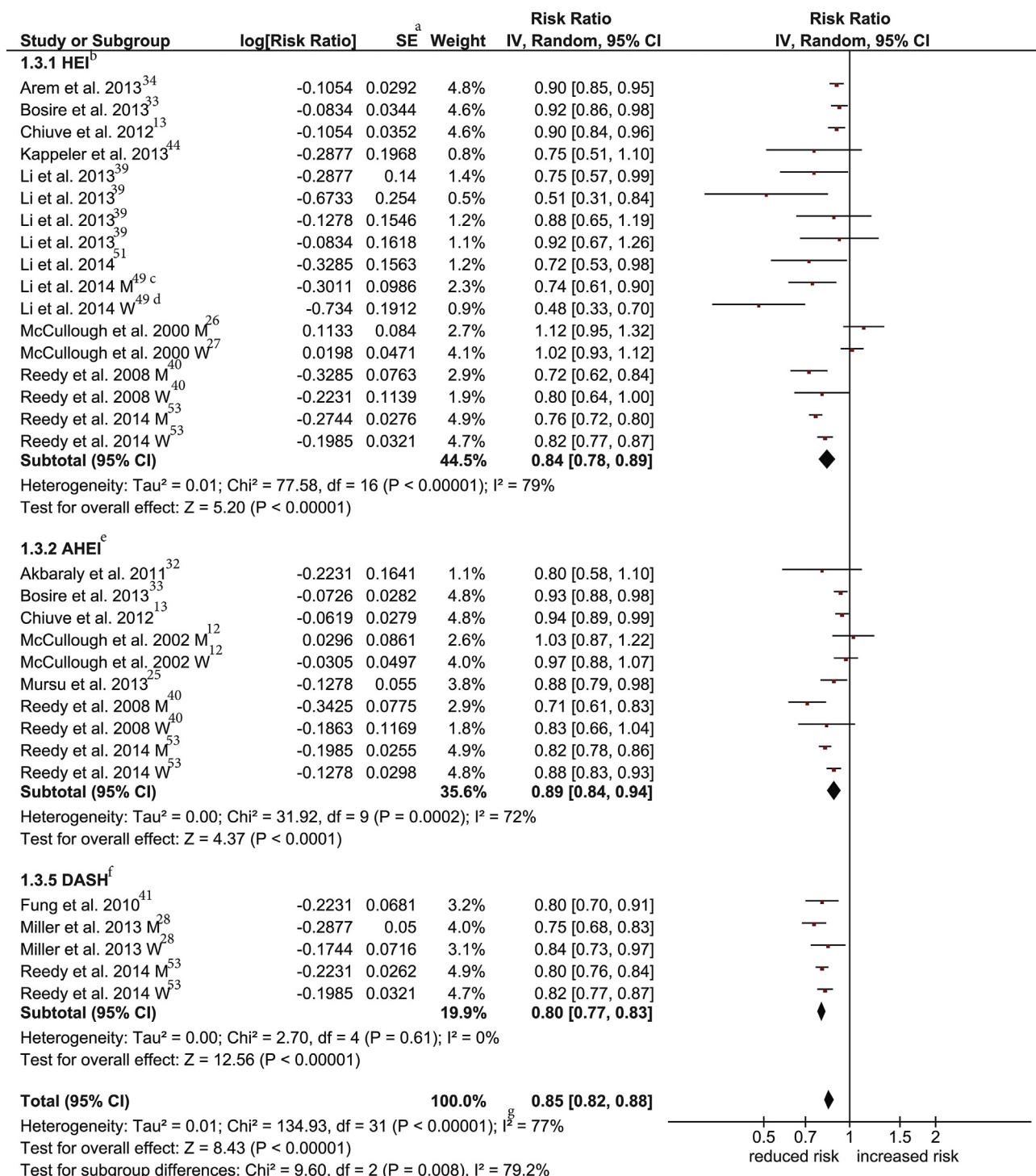


Figure 6. Forest plot showing pooled relative risks (RRs) with 95% CI for the highest diet quality (Healthy Eating Index [HEI], Alternate Health Eating Index [AHEI], and Dietary Approaches to Stop Hypertension [DASH]) vs lowest diet quality category for cancer mortality or incidence. ^aSE=standard error. ^bIncludes the original version, HEI-2005, and HEI-2010. ^cMen. ^dWomen. ^eIncludes the original version, and AHEI-2010. ^fDASH score. ^gI²=inconsistency.

mentioned as the diet's major characteristic and is well established because high blood pressure is ranked as the strongest risk factor attributable to burden of disease.^{18,54} In addition, high salt intake was associated with risk of gastric cancer, albeit not in the United States.⁵⁵

The synergistic effect of individual components may give healthy diets their beneficial properties in preventing type 2 diabetes, as observed in our meta-analysis (including six cohort studies). Meta-analysis of cohort studies has shown that several healthy dietary patterns

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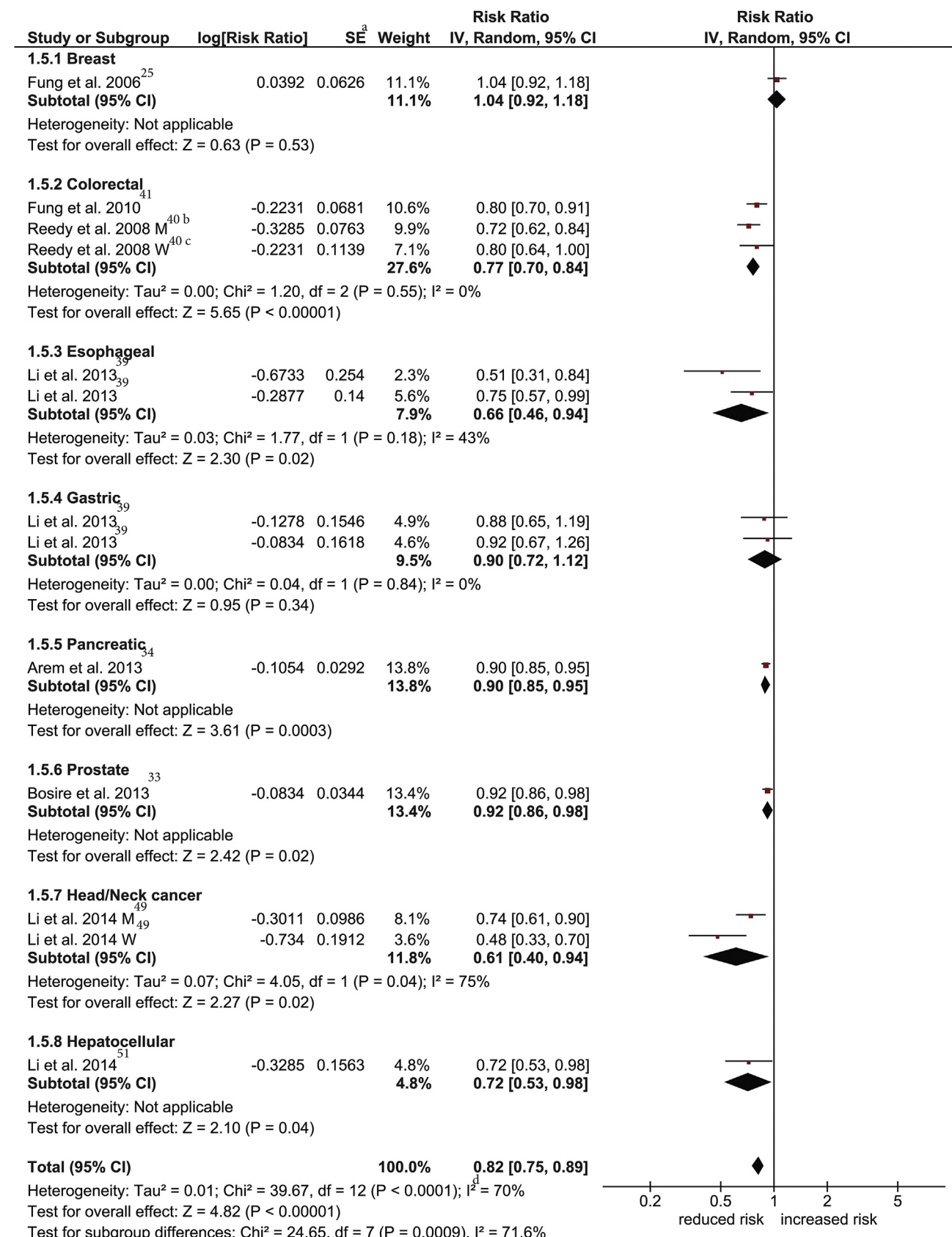


Figure 7. Forest plot showing pooled relative risks (RRs) for the highest diet quality (Healthy Eating Index [HEI], Alternate Health Eating Index [AHEI], and Dietary Approaches to Stop Hypertension [DASH]) vs lowest diet quality category with 95% CI for different cancer types. ^aSE=standard error. ^bMen. ^cWomen. ^dI²=inconsistency.

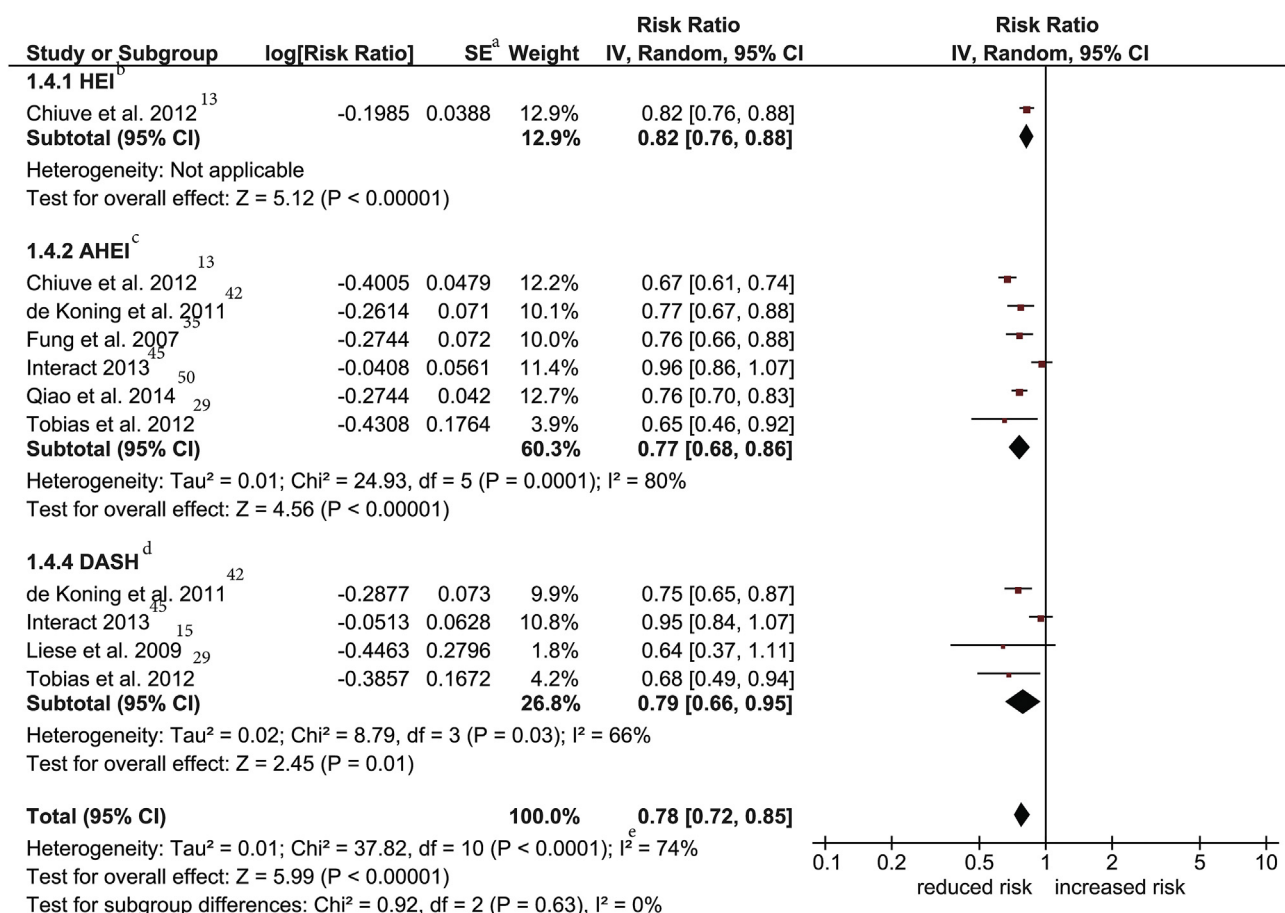


Figure 8. Forest plot showing pooled relative risks (RRs) with 95% CI for the highest diet quality (Healthy Eating Index [HEI], Alternate Health Eating Index [AHEI], and Dietary Approaches to Stop Hypertension [DASH]) vs lowest diet quality category for type 2 diabetes mellitus. ^aSE=standard error. ^bIncludes the original version, HEI-2005, and HEI-2010. ^cIncludes the original version and AHEI-2010. ^dDASH score. ^eI²=inconsistency.

were associated with a significant reduction in type 2 diabetes.⁶⁶ Another point that should be noted is the recommendation of moderate alcohol consumption in the AHEI score. The HEI-2005 grouped alcoholic beverages with calories from solid fats and added sugars. No information regarding alcohol was established by the original HEI and DASH dietary pattern. According to a World Cancer Research Fund 2007 report,⁶⁷ alcoholic beverages providing ≥ 30 g ethanol/day are associated with increased risks of different types of cancer (eg, of the pharynx, larynx, esophagus, colorectal, breast, and liver). A recent meta-analysis of 84 cohort studies of alcohol consumption and cardiovascular disease indicated that alcohol consumption at 2.5 to 14.9 g/day (about ≤ 1 drink a day) was consistently associated with a 25% reduction in the risk of cardiovascular mortality, compared with abstaining from alcohol.⁶⁸

No significant risk reduction could be observed for neurodegenerative diseases. This observation is contrary to the results of a Mediterranean diet meta-analysis by Sofi and colleagues,⁶⁹ who observed a 13% reduction in neurodegenerative diseases associated with a 2-point increase in Mediterranean diet score.

Strengths and Limitations

Several limitations of our systematic review should be considered. Substantial heterogeneity could be observed for all-cause mortality, cancer mortality or incidence, and risk of type 2 diabetes, and confirmed through the sensitivity analysis. Potential sources of heterogeneity include differences in how the dietary quality indexes were defined and scored, heterogeneous risk estimates, as well as heterogeneous populations/age/sex, sample sizes, and follow-ups. The HEI, the AHEI, the DASH score, and the corresponding updates are heterogeneous with respect to how the components were scored (ie, HEI based on absolute values, and the HEI-2005/2010 was based on amounts per 1,000 kcal; AHEI and DASH score were based on absolute values). All diets that scored high on the diet quality indexes included in our meta-analysis showed a reduced risk of CVD and type 2 diabetes. However, only diets that scored highly on the updated HEI-2005/2010 and AHEI-2010 were associated with a reduced risk of cancer. Thus, the results of our meta-analysis indicate that the modifications that were made when creating the more recent versions of the HEI and AHEI were associated with reduced risk of cancer. Another limitation is the fact that cohort studies have caveats, including a reliance on

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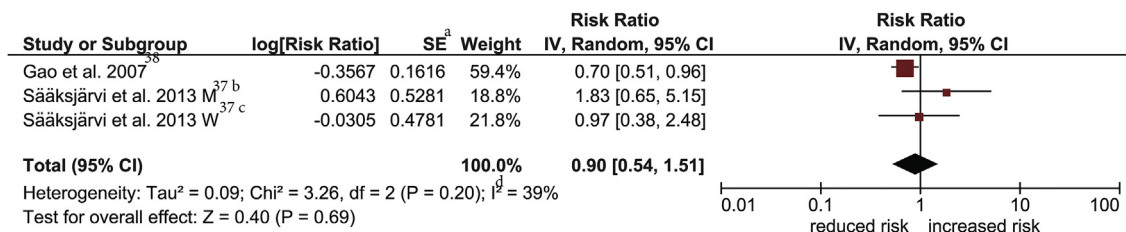


Figure 9. Forest plot showing pooled relative risks (RRs) with 95% CI for the highest diet quality (Healthy Eating Index [HEI], Alternate Health Eating Index [AHEI], and Dietary Approaches to Stop Hypertension [DASH]) vs lowest diet quality category for Parkinson's disease. ^aSE=standard error. ^bMen. ^cWomen. ^dI²=inconsistency.

Table 2. Relative risk (95% CI) of the association of diet quality as assessed by the Healthy Eating Index (HEI), alternate Healthy Eating Index (AHEI), or Dietary Approaches to Stop Hypertension (DASH) score for all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, cancer types, type 2 diabetes, and Parkinson's disease

Outcome	No. of studies	Index	Relative risk	95% CI	P value	I ² (%) ^a (95% CI)
All-cause mortality	4	All indexes combined	0.78	0.76-0.80	<0.00001	61 (20-81)
	2	HEI	0.78	0.76-0.79	<0.00001	0
	3	AHEI	0.77	0.75-0.79	<0.00001	42
	1	DASH	0.80	0.76-0.86	<0.00001	81
Cardiovascular mortality or incidence	17	All indexes combined	0.78	0.75-0.81	<0.00001	45 (13-66)
	6	HEI	0.82	0.79-0.85	<0.00001	0
	6	AHEI	0.74	0.72-0.77	<0.00001	10
	9	DASH	0.80	0.76-0.85	<0.00001	30
Cancer mortality or incidence	16	All indexes combined	0.85	0.82-0.88	<0.00001	77 (68-84)
	11	HEI	0.84	0.78-0.89	<0.00001	79
	7	AHEI	0.89	0.84-0.94	<0.0001	72
	3	DASH	0.80	0.77-0.83	<0.00001	0
Breast cancer	1	All indexes combined	1.04	0.92-1.18	0.53	N/A ^b
Colorectal cancer	2	All indexes combined	0.77	0.70-0.84	<0.00001	0
Esophageal cancer	1	All indexes combined	0.66	0.46-0.94	0.02	43
Gastric cancer	1	All indexes combined	0.90	0.72-1.12	0.34	0
Pancreatic cancer	1	All indexes combined	0.90	0.85-0.95	0.0003	N/A
Prostate cancer	1	All indexes combined	0.92	0.86-0.98	0.02	N/A
Head and neck cancer	1	All indexes combined	0.61	0.40-0.94	0.02	75
Hepatocellular carcinoma	1	All indexes combined	0.72	0.53-0.98	0.04	N/A
Type 2 diabetes mellitus	7	All indexes combined	0.78	0.72-0.85	<0.00001	74 (52-86)
	1	HEI	0.82	0.76-0.88	<0.00001	N/A
	6	AHEI	0.77	0.68-0.86	<0.00001	80
	4	DASH	0.79	0.66-0.95	0.01	66
Parkinson's disease	2	AHEI	0.90	0.54-1.51	0.69	39 (0-81)

^aI²=Inconsistency, percentage of variation across studies due to heterogeneity.

^bN/A=not applicable.

nutritional assessment methods with validity and reliability being lower compared with randomized controlled trials, because dietary intake is usually calculated from self-assessment tools such as food frequency questionnaires.⁷⁰ However, compared with case-control studies, prospective cohort studies have a lower risk of differential recall bias (no differential response between cases and controls) and measurement errors (ie, information on health status not typically collected by self-report, interview, or from recorded information).⁷¹ A further limitation exists in the multivariate adjustments for potential confounders in each study. Whereas almost all studies were adjusted for age, sex, body mass index, energy intake, education, and physical activity, several studies were not adjusted for dyslipidemia, hypertension, family history of CVD, alcohol intake, aspirin, or postmenopausal hormone use. In addition, the AHEI used by Skäåksjärvi and colleagues³⁷ did not include multivitamin use and alcohol consumption in the score, probably affecting the results on the neurodegenerative outcome parameter. In our meta-analysis, heterogeneous risk estimates were included (20 reports provided HR, 13 reports provided RR, and one report provided odds ratio). RR and odds ratio depict only the number of events, not taking into account the exact time-point of occurrence. These indexes are appropriate for measuring dichotomous outcomes, but less appropriate for analyzing time-to-event outcomes. Therefore, combining HR, RR, and odds ratio reported at different stages of maturity (with variable follow-up), might result in an estimate that is both unreliable and difficult to interpret.⁷²

The vast majority of meta-analyses in the Cochrane Database of Systematic Reviews often underestimate existing heterogeneity or fail to identify it completely.⁷³ In addition, in small meta-analyses the standard models are underperforming and both coverage (Type 1 error) and power (Type 2 error) are affected.⁷⁴ However, a meta-analysis of two studies can still be informative despite these problems. To account for the heterogeneity uncertainty we repeated the analyses assuming high levels of heterogeneity using the *metaan* command in Stata²⁴ and our results and conclusions did not change, which gives us more confidence regarding the validity of our findings.

Our systematic review has several strengths, as well. Many of the studies, especially cohort studies, were of high methodologic quality. Overall, more than 1 million subjects were included in the meta-analysis, providing the power to detect statistically significant RR as well as assess publication bias.

CONCLUSIONS

Our systematic review and meta-analysis shows that high-quality diets, as assessed by the HEI, AHEI, and DASH scores, are associated with a reduced risk of all-cause mortality, manifestation of type 2 diabetes, and incidence or mortality due to cardiovascular disease and overall cancer, particularly colorectal cancer. This underlines the relevance of these indexes in clinical as well as public health settings.

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STATEMENT OF POTENTIAL CONFLICT OF INTEREST

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Keywords: (*Healthy Eating Index*) (*DASH diet*)

healthy [All Fields] AND (*eating* [MeSH Terms] OR *eating* [All Fields]) AND (*abstracting and indexing as topic* [MeSH Terms] OR (*abstracting* [All Fields] AND *indexing* [All Fields] AND *topic* [All Fields]) OR *abstracting and indexing as topic* [All Fields] OR *index* [All Fields]) *dash* [All Fields] AND (*diet* [MeSH Terms] OR *diet* [All Fields])

Figure 1. Search strategy.

Selection

- 1) Representativeness of the exposed cohort
 - a) truly representative of the average _____ (describe) in the community *
 - b) somewhat representative of the average _____ in the community *
 - c) selected group of users (eg, nurses, volunteers)
 - d) no description of the derivation of the cohort
- 2) Selection of the nonexposed cohort
 - a) drawn from the same community as the exposed cohort *
 - b) drawn from a different source
 - c) no description of the derivation of the nonexposed cohort
- 3) Ascertainment of exposure
 - a) secure record (eg, surgical records) *
 - b) structured interview *
 - c) written self-report
 - d) no description
- 4) Demonstration that outcome of interest was not present at start of study
 - a) yes *
 - b) no

Comparability

- 1) Comparability of cohorts on the basis of the design or analysis
 - a) study controls for _____ (select the most important factor) *
 - b) study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

- 1) Assessment of outcome
 - a) independent blind assessment *
 - b) record linkage *
 - c) self-report
 - d) no description
- 2) Was follow-up long enough for outcomes to occur
 - a) yes (select an adequate follow-up period for outcome of interest) *
 - b) no
- 3) Adequacy of follow up of cohorts
 - a) complete follow-up - all subjects accounted for *
 - b) subjects lost to follow-up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow-up, or description provided of those lost) *
 - c) follow-up rate < ____% (select an adequate %) and no description of those lost
 - d) no statement

Figure 2. Newcastle-Ottawa Quality Assessment Scale for cohort studies. *A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability. Reprinted with permission from George A. Wells, MSc, PhD.

Table 3. US cohort studies: Relative risk and 95% CI of the association of diet quality as assessed by the Healthy Eating Index, alternate Healthy Eating Index (AHEI), or Dietary Approaches to Stop Hypertension score for all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, type 2 diabetes, and Parkinson's disease

Outcome	Data points	Index	Relative risk	95% CI	P value	I ² (%) ^a
All-cause mortality	4	All indexes combined	0.78	0.76-0.80	<0.00001	66
Cardiovascular mortality or incidence	14	All indexes combined	0.79	0.76-0.81	<0.00001	47
Cancer mortality or incidence	20	All indexes combined	0.85	0.82-0.88	<0.00001	77
Type 2 diabetes mellitus	6	All indexes combined	0.75	0.71-0.79	<0.00001	35
Parkinson's disease	1	AHEI	0.70	0.51-0.96	0.03	N/A ^b

^aI²=inconsistency, percentage of variation across studies due to heterogeneity.

^bN/A=not applicable.

Table 4. Long-term follow-up (≥ 8 years): Relative risk and 95% CI of the association of diet quality as assessed by the Healthy Eating Index, alternate Healthy Eating Index (AHEI), or Dietary Approaches to Stop Hypertension score for all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, type 2 diabetes, and Parkinson's disease

Outcome	Data points	Index	Relative risk	95% CI	P value	I ² (%) ^a
All-cause mortality	5	All indexes combined	0.78	0.76-0.80	<0.00001	61
Cardiovascular mortality or incidence	17	All indexes combined	0.78	0.75-0.81	<0.00001	43
Cancer mortality or incidence	18	All indexes combined	0.85	0.82-0.89	<0.00001	78
Type 2 diabetes mellitus	6	All indexes combined	0.79	0.73-0.85	<0.00001	76
Parkinson's disease	3	AHEI	0.90	0.54-1.51	0.69	39

^aI²=inconsistency, percentage of variation across studies due to heterogeneity.

Table 5. High-quality studies (≥ 7 points Newcastle-Ottawa Quality Assessment Scale): Relative risk and 95% CI of the association of diet quality as assessed by the Healthy Eating Index, alternate Healthy Eating Index (AHEI), or Dietary Approaches to Stop Hypertension score for all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, type 2 diabetes, and Parkinson's disease

Outcome	Data points	Index	Relative risk	95% CI	P value	I^2 (%) ^a
All-cause mortality	4	All indexes combined	0.78	0.76-0.80	<0.00001	66
Cardiovascular mortality or incidence	17	All indexes combined	0.78	0.75-0.81	<0.00001	43
Cancer mortality or incidence	20	All indexes combined	0.85	0.82-0.88	<0.00001	77
Type 2 diabetes mellitus	5	All indexes combined	0.83	0.73-0.87	<0.00001	80
Parkinson's disease	1	AHEI	0.70	0.51-0.96	0.03	N/A ^b

^a I^2 =inconsistency, percentage of variation across studies due to heterogeneity.

^bN/A=not applicable.

Table 6. Singular cohorts: Relative risk and 95% CI of the association of diet quality as assessed by the Healthy Eating Index, alternate Healthy Eating Index (AHEI), or Dietary Approaches to Stop Hypertension score for all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, type 2 diabetes, and Parkinson's disease

Outcome	Data points	Index	Relative risk	95% CI	P value	I^2 (%) ^a
All-cause mortality	5	All indexes combined	0.78	0.76-0.80	<0.00001	66
Cardiovascular mortality or incidence	15	All indexes combined	0.79	0.76-0.81	<0.00001	45
Cancer mortality or incidence	13	All indexes combined	0.84	0.80-0.87	<0.00001	76
Type 2 diabetes mellitus	6	All indexes combined	0.80	0.72-0.88	<0.00001	81
Parkinson's disease	3	AHEI	0.90	0.54-1.51	0.69	39

^a I^2 =inconsistency, percentage of variation across studies due to heterogeneity.

Table 7. Women: Relative risk and 95% CI of the association of diet quality as assessed by the Healthy Eating Index, alternate Healthy Eating Index (AHEI), or Dietary Approaches to Stop Hypertension score for all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, type 2 diabetes, and Parkinson's disease

Outcome	Data points	Index	Relative risk	95% CI	P value	I ² (%) ^a
All-cause mortality	2	All indexes combined	0.78	0.76-0.80	<0.00001	41
Cardiovascular mortality or incidence	10	All indexes combined	0.77	0.75-0.80	<0.00001	17
Cancer mortality or incidence	7	All indexes combined	0.86	0.81-0.92	<0.00001	73
Type 2 diabetes mellitus	3	All indexes combined	0.75	0.70-0.80	<0.00001	0
Parkinson's disease	1	AHEI	0.97	0.38-2.48	0.95	N/A ^b

^aI²=inconsistency, percentage of variation across studies due to heterogeneity.

^bN/A=not applicable.

Table 8. Men: Relative risk and 95% CI of the association of diet quality as assessed by the Healthy Eating Index, alternate Healthy Eating Index (AHEI), or Dietary Approaches to Stop Hypertension score for all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, type 2 diabetes, and Parkinson's disease

Outcome	Data points	Index	Relative risk	95% CI	P value	I ² (%) ^a
All-cause mortality	1	All indexes combined	0.79	0.75-0.83	<0.00001	86
Cardiovascular mortality or incidence	4	All indexes combined	0.79	0.73-0.85	<0.00001	74
Cancer mortality or incidence	7	All indexes combined	0.84	0.79-0.90	<0.00001	85
Type 2 diabetes mellitus	1	All indexes combined	0.76	0.69-0.84	<0.00001	0
Parkinson's disease	1	AHEI	1.83	0.65-5.15	0.25	N/A ^b

^aI²=inconsistency, percentage of variation across studies due to heterogeneity.

^bN/A=not applicable.

RESEARCH

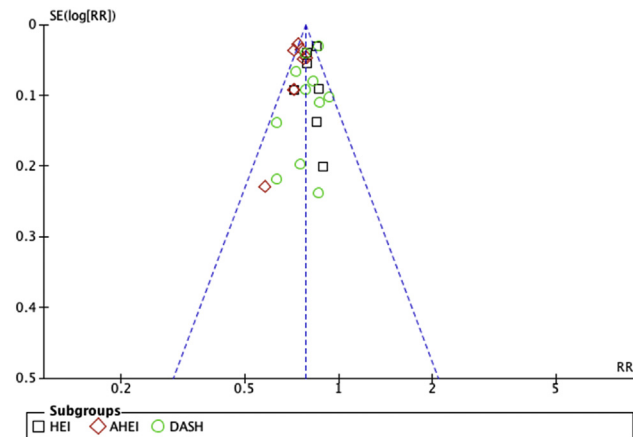


Figure 10. Funnel plot showing study precision against the relative risk (RR) with 95% CIs for cardiovascular mortality or incidence. SE=standard error. HEI=Healthy Eating Index. AHEI=Alternate Health Eating Index. DASH=Dietary Approaches to Stop Hypertension.

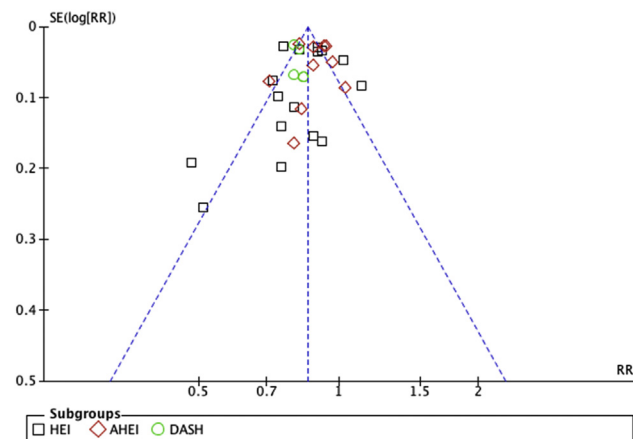


Figure 11. Funnel plot showing study precision against the relative risk (RR) with 95% CIs for cancer mortality or incidence. SE=standard error. HEI=Healthy Eating Index. AHEI=Alternate Health Eating Index. DASH=Dietary Approaches to Stop Hypertension.

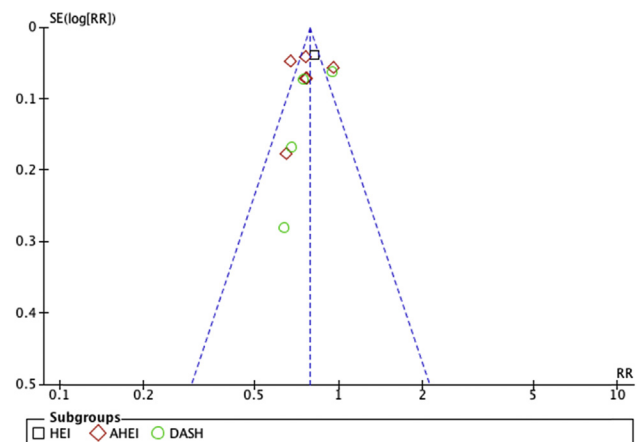


Figure 12. Funnel plot showing study precision against the relative risk with 95% CIs type 2 diabetes mellitus. SE=standard error. HEI=Healthy Eating Index. AHEI=Alternate Health Eating Index. DASH=Dietary Approaches to Stop Hypertension.

Impact of Different Training Modalities on Anthropometric and Metabolic Characteristics in Overweight/Obese Subjects: A Systematic Review and Network Meta-Analysis

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Abstract

Background: The aim of this systematic review of randomized controlled trials was to compare the effects of aerobic training (AET), resistance training (RT), and combined aerobic and resistance training (CT) on anthropometric parameters, blood lipids, and cardiorespiratory fitness in overweight and obese subjects.

Methods: Electronic searches for randomized controlled trials were performed in MEDLINE, EMBASE and the Cochrane Trial Register. Inclusion criteria were: Body Mass Index: ≥ 25 kg/m², 19+ years of age, supervised exercise training, and a minimum intervention period of 8 weeks. Anthropometric outcomes, blood lipids, and cardiorespiratory fitness parameters were included. Pooled effects were calculated by inverse-variance random effect pairwise meta-analyses and Bayesian random effects network meta-analyses.

Findings: 15 trials enrolling 741 participants were included in the meta-analysis. Compared to RT, AET resulted in a significantly more pronounced reduction of body weight [mean differences (MD): -1.15 kg, $p=0.04$], waist circumference [MD: -1.10 cm, $p=0.004$], and fat mass [MD: -1.15 kg, $p=0.001$] respectively. RT was more effective than AET in improving lean body mass [MD: 1.26 kg, $p<0.00001$]. When comparing CT with RT, MD in change of body weight [MD: -2.03 kg, $p<0.0001$], waist circumference [MD: -1.57 cm, $p=0.0002$], and fat mass [MD: -1.88 kg, $p<0.00001$] were all in favor of CT. Results from the network meta-analyses confirmed these findings.

Conclusion: Evidence from both pairwise and network meta-analyses suggests that CT is the most efficacious means to reduce anthropometric outcomes and should be recommended in the prevention and treatment of overweight, and obesity whenever possible.

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Background

Recent data provided by the World Health Organization illustrates that the global prevalence of overweight and obesity has more than doubled since 1980. In 2008, more than 1.4 billion adults aged 20 and older were overweight with over 500 million of them being obese [1]. Since overweight and obesity are evidence-based risk factors for diabetes, cardiovascular disease (CVD) or cancer, [2] the emergence of obesity as a pandemic represents a serious challenge for public health authorities. Exercise and diet are established cornerstones in the primary prevention as well as in the management of obesity [3]. Thus, the American College of Sports Medicine and the American Heart Association both recommend either moderate-intensity aerobic physical activity for a minimum of 30 min on five days each week or vigorous-intensity aerobic activity for a minimum of 20 min on three days each week for healthy adults. In addition, these authorities

encourage regular resistance training (RT) for a minimum of two days per week performing 8 exercises with 8–12 repetitions [4].

Several meta-analyses investigated the independent effects of aerobic exercise training (AET) on anthropometric and cardio-metabolic risk factors, providing evidence for reductions in body mass index (BMI), body weight (BW), waist circumference (WC), visceral adipose tissue, and increasing high-density lipoprotein cholesterol (HDL-C) and maximum oxygen uptake (VO₂max) [5–9]. With respect to RT, some meta-analyses reported reductions of HbA1c, systolic blood pressure (SBP), diastolic blood pressure (DBP), and C-reactive protein (CRP) [10–12].

The above mentioned meta-analyses were performed in order to compare one or more of the training modalities with the data from a sedentary control group. To date, no systematic review has pooled the effects of different training modalities on

anthropometrical and cardiovascular risk factors. Therefore, the aim of the present study was to conduct a systematic review and meta-analysis of randomized controlled trials (RCTs) to assess the efficacy of AET, RT, and CT on anthropometric outcomes, blood lipids, and cardiorespiratory fitness in overweight and obese subjects.

Methods

The review protocol was registered in PROSPERO International Prospective Register of Systematic Reviews (crd.york.ac.uk/prospero/index.asp Identifier: CRD42013003905).

Literature Search

Queries of literature were performed using the electronic databases MEDLINE (between 1966 and December 2012), EMBASE (between 1980 and December 2012), and the Cochrane Trial Register (until December 2012) restricted to randomized controlled trials and quasi-randomized controlled trials, but no restrictions to calendar date. The following search terms were used: (“*strength AND training*”; “*resistance AND training*”; “*aerobic AND training*”; “*exercise AND training*”; “*endurance AND training*”). Moreover, the reference lists from retrieved articles, systematic reviews, and meta-analyses were checked to search for further relevant studies. This systematic review was planned, conducted, and reported in adherence to standards of quality for reporting meta-analyses [13]. Literature search was conducted independently by two authors, with disagreements resolved by consensus.

Eligibility Criteria

Studies were included in the meta-analysis if they met all of the following criteria: (1) randomized controlled design; (2) minimum intervention period of 8 weeks; (3) body mass index ≥ 25 kg/m²; (4) age: ≥ 19 years; (5) comparison of either AET vs. RT and/or CT vs. AET and/or CT vs. RT; (6) assessment of “primary outcome” markers: BW, WC, waist to hip ratio (WHR), fat mass (FM, given in kg), lean body mass (LBM, given in kg); assessment of “secondary outcome” markers: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C, triacylglycerols (TG) and VO₂ max; (7) report of post-treatment mean values (if not available mean of changes from baseline were used) with standard deviation (or data suitable to calculate these parameters: standard error, 95% confidence interval); (8) training had to be supervised, not home-based; (9) exclusion of studies with a dietary

co-intervention that was not applied in all intervention groups; (10) exclusion of subjects with type 2 diabetes, and coronary heart disease.

All abstracts and full text were assessed for eligibility independently by two authors.

Risk of Bias Assessment

Full copies of studies were independently assessed by two authors for methodological quality using the risk of bias assessment tool by the Cochrane Collaboration [14,15]. The following sources of bias were detected: selection bias (random sequence generation, allocation concealment), performance/detection bias (blinding of outcome assessment), attrition bias (incomplete data outcome), reporting bias (selective reporting) and other bias (in this case, trials were assessed for risk of bias in relation to “systematic difference in care”) (Figure 1).

Data Extraction and statistical analysis

The following data were extracted from each study: the first author’s last name, publication year, study duration, participant’s sex and age, BMI, sample size, treatment effects, intervention type, dose, intensity and frequency, post mean values or differences in mean of two time point values with corresponding standard deviation. For each outcome measure of interest, pairwise and network random-effects meta-analyses were performed in order to determine the pooled relative effect of each intervention relative to every other, in terms of mean differences (MDs) between the post-intervention (or change from baseline differences in means) values of the different interventions. Combining both the post-intervention values and difference in means in one meta-analysis is a legitimate method described by the Cochrane Collaboration [15], which assumes that the relative effects estimated by both the post-intervention and the mean change from baseline measures is the same. However an additional assessment of baseline comparability was done and is summarized in Table S2 in File S1. Data were pooled if outcomes were reported by at least three studies. Heterogeneity between trial results was tested with a Cochran’s Q test. A value for $I^2 > 50\%$ was considered to represent substantial heterogeneity [16]. To consider heterogeneity, the random-effects model was used to estimate MDs with 95% confidence intervals (CIs). Forest plots were generated to illustrate the study-specific effect sizes along with a 95% CI. To determine the presence of publication bias, the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard

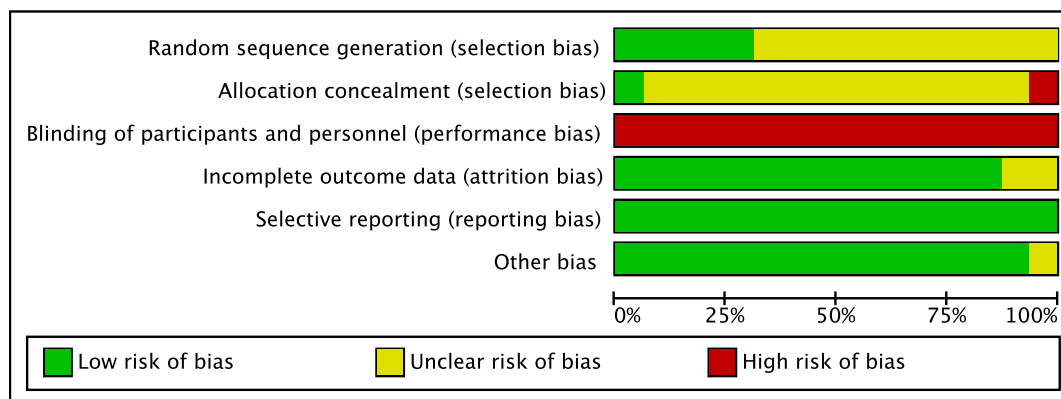


Figure 1. Risk of bias assessment tool. Across trials ($n = 15$), information is either from trials at a low risk of bias (green), or from trials at unclear risk of bias (yellow), or from trials at high risk of bias (red).
doi:10.1371/journal.pone.0082853.g001

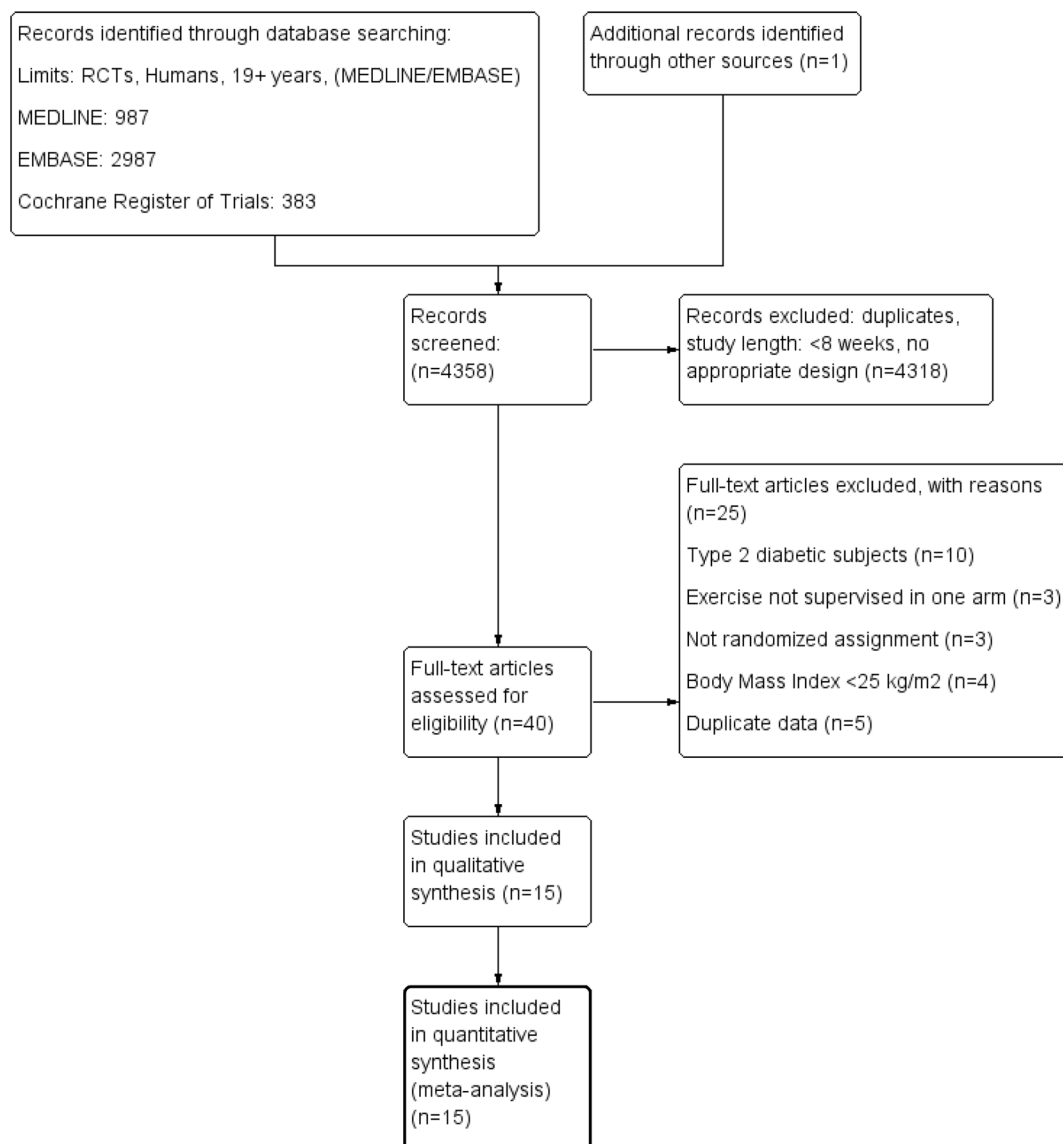


Figure 2. Flow diagram.

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errors were assessed. Additionally, Begg's and Egger regression tests were performed to detect small study effects [17,18].

Pooled effect sizes from the network meta-analyses are presented as posterior medians and 95% credible intervals (CrI) (i.e. Bayesian equivalent of confidence intervals) in the appropriate units. Separate pairwise meta-analyses were first used to compare all interventions. Network meta-analysis was then used to synthesize all the available evidence [19]. Network meta-analysis methods are extensions of the standard pairwise meta-analysis model which enable simultaneous comparison of multiple interventions whilst preserving the internal randomization of individual trials. They have the advantage of adequately accounting for the correlation in relative effect estimates from 3-arm trials as well as providing a single coherent summary of all the evidence. For pairwise meta-analyses, data were analyzed using the REVIEW MANAGER 5.1 software, provided by the Cochrane Collaboration (<http://ims.cochrane.org/revman>). Network meta-analyses were conducted using Markov chain Monte Carlo (MCMC) simulation implemented through the freely

available software WINBUGS, version 1.4.3 [20]. The WINBUGS code used is freely available online [19,21] (program "TSD2-5aRE_Normal_id.odc").

Minimally informative normal priors were used for all treatment effect parameters and a Uniform (0,150) prior was used for the between-study standard deviation (heterogeneity) parameter. Sensitivity to this prior was assessed, but there was no meaningful change in relative effects or overall conclusions.

Three MCMC chains were used to assess convergence using Brooks-Gelman-Rubin plots and by inspection of the trace plots [22]. Convergence was achieved after 20,000 iterations for all outcomes. Posterior summaries were then obtained from further simulation of 50,000 iterations in each of the 3 chains (150,000 in total), resulting in a small Monte Carlo error.

The potential for inconsistency was assessed by inspection of the available evidence. In case of possible inconsistency, Bayesian p-values for the difference between direct and indirect evidence were calculated, and direct and indirect estimates were compared [23,24].

Table 1. General study characteristics.

Reference	Sample size, Mean baseline BMI (kg/m ²)	Mean age (yrs)	Duration (months)	Study design	Exercise prescription	Outcomes (*significant changes between groups) p<0.05
Ahmadizad et al. 2007 [30]	16	40.9	3	RT vs.	RT: 11 Ex, 50–60% 1RM, dose: 12 S/MG/W;	RT: ↑ VO ₂ max
	28.1	0%		AET	AET: 75–85% MHR, 60–90 min/wk;	AET: ↑ VO ₂ max
Ballor et al. 1996 [39]	18	61	3	RT vs.	RT: 7 Ex, 50–80% 1 RM, 8 R, 9 S/MG/W;	RT: ↑ LBM
	>32	55%		AET	AET: 50% VO ₂ max, 60–180 min/wk;	AET: ↓ BW, FM; ↑ VO ₂ max
Banz et al. 2001 [31]	19	47.5	2.5	RT vs.	RT: 8 Ex, 10 R, dose: 9 S/MG/W;	RT: ↓ WHR;
	33	0%		AET	AET: 60–85% MHR, 120 min/wk;	AET: ↓ WHR; ↑ HDL-C
Bateman et al. 2011 [32]	196	49.5	4	RT vs.	RT: 8 Ex, 8–12 R, dose: 9 S/MG/W;	RT: ↑ BW, LBM; ↑ VO ₂ max
	Willis et al. 2012 [38]					
	30.5	48%		AET vs.	AET: 65–80% VO ₂ max; 130 min/wk;	AET: ↓ BW, WC, FM, TG; ↑ VO ₂ max
		52%		CT	CT: RT: 8 Ex, 8–12 R, dose: 9 S/MG/W; RT: 65–80% VO ₂ max; 130 min/wk;	CT: ↓ BW, WC, FM, TG; ↑ LBM, VO ₂ max
Davidson et al. 2009 [33]	108	67.7	6	RT vs.	RT: 9 Ex, dose: 3 S/MG/W;	RT: ↓ WC ^a
	30	57%		AET vs.	AET: 60–75% VO ₂ max, 150 min/wk;	AET: ↓ BW ^{a,b} , WC ^{a,b} , FM ^{a,b} ; ↑ VO ₂ max ^{a,b}
		43%		CT	CT: RT: 9 Ex, 3 S/MG/W, AET: 60–75% VO ₂ max, 150 min/wk;	CT: ↓ BW ^{a,b} , WC ^a , FM ^{a,b} ; ↑ VO ₂ max ^{a,b} *compared with control ^a , RT ^b ,
Donges et al. 2010 [34]	76	n.d	2.5	RT vs.	RT: 6 Ex, 75% 1RM, dose: 3 S/MG/W;	RT: ↓ WC ↑ BW, LBM
	27.8	58%		AET	AET: 75% MHR, 150 min/wk;	AET: ↓ BW, WC, FM; ↑ LBM
Fenkci et al. 2006 [37]	40	42.85	4	RT vs.	RT: 6 Ex, 75–80% 1 RM, 10 R, dose: 9 S/MG/W;	RT: ↓ BW, FM; ↑ LBMAET: ↓ BW, FM
	Sarsan et al. 2006 [47]					
	35	100%		AET	AET: 50–85% HRR, 45–225 min/wk;	AET: ↓ BW, FM
		0%				
Fisher et al. 2011 [40]	97	30.5	until BMI <25 kg/m	RT + CR vs.	RT: 10 Ex, 80% 1 RM, 10 R, dose: 6 S/MG/W;	RT: ↓ BW, WC, TC, TG
	28	100%		AET + CR	AET: 65–80% MHR, 150 min/wk;	AET: ↓ BW, WC, TC, TG
		0%				
Janssen et al. 2002 [35]	25	36.15	4	RT + CR vs.	RT: 7 Ex, 8–12 R, dose: 3 S/MG/W;	RT: ↓ BW, WHR, WC, TC, LDL-C, HDL-C
	33.8	100%		AET + CR	AET: 50–85% of MHR, max. 300 min/wk;	AET: ↓ BW, WHR, WC, TC
Martins et al. 2010 [36]	32	73.2	4	RT vs.	RT: 8 Ex, 8–15 RM, dose: 3–9 S/MG/W;	RT: /

Table 1. Cont.

Reference	Sample size, Mean age (yrs)	Duration (months)	Study design	Exercise prescription	Outcomes (*significant changes between groups) p<0.05
	Mean baseline BMI (kg/m ²)	Female (%)			
		Male (%)			
	30.8	63%	AET	AET: 40–85% HRR, 120 min/wk;	AET: ↓ BW, WC, LDL-C
		37%			
Potteiger et al. 2012 [29]	22	36.4	RT vs.	RT: high: 100% 5–7 RM, moderate: 80% 8–10 RM, dose: increase from 3 to max 16 S/MG/W; AET: 65–80%VO ₂ max; 135–180 min/wk;	RT: ↓ HDL-C, WC, FM; ↑ LBMAET: ↓ BW, WC, TG
	31.2	0%	AET		AET: ↓ BW, WC, TG
		100%			
Rice et al. 1999 [28]	20	43.6	RT +CR vs.	RT: 7 Ex; 8–12 R, dose: 3 S/MG/W;	RT: ↓ BW, WHR, WC
	33.05	0%	AET +CR	AET: 50–85% of MHR, max 300 min/wk;	AET: ↓ BW, WHR, WC
		100%			
Ross et al. 1994 [27]	24	36.1	RT +CR vs.	RT: 8 Ex, 70–80% 1RM, dose: 3 S/MG/W;	RT: ↓ BW, WC
	33.1	100%	AET +CR	AET: 50–85% MHR, 45–180 min/wk;	AET: ↓ BW, WC
		0%			
Stensvold et al. 2010 [26]	32	51.23	RT vs.	RT: 15–20 R, dose: 6–9 S/MG/W;	RT: ↓ WC, FM
	31.26	40%	AET vs.	AET: interval training (90–95% VO ₂ max), 130 min/wk;	AET: ↓ WC, FM
		60%	CT	CT: AET (2x wk), RT (1x wk);	CT: ↓ WC; ↑ LBM
Wallace et al. 1997 [25]	16	41.2	AET vs.	AET: 60–70% HRR, 180 min/wk;	AET: ↑ VO ₂ max,
	94 kg	0%	CT	CT: RT: 8 Ex, 75% 1RM, 8–12 R, dose: 12 S/MG/W; AET: 60–70% HRR, 180 min/wk;	CT: ↓ FM, TG; ↑ HDL-C, VO ₂ max
		100%			

AET, aerobic endurance training; BW, body weight; CR, caloric restriction; CT, combined training (RT and AET); Ex, exercises; FM, fat mass; HDL-C, high-density lipoprotein cholesterol; HRR, heart rate reserve; LBM, lean body mass; LDL-C, low-density lipoprotein cholesterol; MHR, maximum heart rate; n.d., no data; R, Repetition; RT, resistance training; S/MG/W, sets for each muscle group per week; TC, total cholesterol; TG, triacylglycerols; VO₂ max, maximal oxygen uptake; WC, waist circumference; WHR, waist to hip ratio; ↑, higher/more; ↓, lower/less.
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Table 2. Pooled estimates of effect size (95% confidence intervals) expressed as weighted mean difference for the effects of AET vs. RT, CT vs. AET and CT vs. RT on anthropometric outcomes, blood lipids and cardiorespiratory fitness.

Outcomes	No. of Studies	Sample Size	MD	95% CI	p-values	Inconsistency I ²	Egger test
AET vs. RT							
BW (kg)	14	560	-1.15	[-2.23, -0.07]	0.04	34%	0.032
WC (cm)	10	410	-1.10	[-1.85, -0.36]	0.004	0%	0.742
WHR	8	232	-0.01	[-0.02, 0.01]	0.48	82%	0.156
FM (kg)	8	415	-1.14	[-1.83, -0.45]	0.001	3%	0.277
LBM (kg)	7	335	-1.26	[-1.81, -0.71]	<0.00001	0%	0.883
TC (mg/dl)	7	230	-2.40	[-10.29, 5.50]	0.55	0%	0.270
LDL-C (mg/dl)	6	208	-3.69	[-14.91, 7.52]	0.52	46%	0.841
HDL-C (mg/dl)	8	291	1.49	[-0.18, 3.16]	0.08	0%	0.203
TG (mg/dl)	7	272	-7.63	[-22.61, 7.34]	0.32	0%	0.481
VO2max (ml/kg/min)	7	260	2.53	[1.62, 3.44]	<0.00001	0%	0.362
CT vs. AET							
BW (kg)	4	184	0.34	[-0.39, 1.08]	0.36	0%	0.141
WC (cm)	3	168	-0.14	[-1.03, 0.76]	0.77	0%	0.688
FM (kg)	4	184	-0.56	[-1.34, 0.22]	0.16	0%	0.234
LBM (kg)	3	112	0.90	[0.31, 1.48]	0.003	0%	0.600
HDL-C (mg/dl)	3	92	0.76	[-1.30, 2.81]	0.47	0%	0.079
TG (mg/dl)	3	92	0.19	[-19.47, 19.86]	0.98	0%	0.297
VO2max (ml/kg/min)	4	172	-0.04	[-1.47, 1.39]	0.96	25%	0.024
CT vs. RT							
BW (kg)	3	173	-2.03	[-2.94, -1.12]	<0.0001	19%	0.400
WC (cm)	3	173	-1.57	[-2.38, -0.75]	0.0002	0%	0.295
FM (kg)	3	173	-1.88	[-2.67, -1.08]	<0.00001	9%	0.297
VO2max (ml/kg/min)	3	162	2.79	[1.78, 3.79]	<0.00001	0%	0.102

BW, body weight; CRP, CRP; FM, fat mass; HDL-C, high density lipoprotein cholesterol; LBM, lean body mass; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triacylglycerols; VO₂ max, maximal oxygen uptake; WC, waist circumference; WHR, waist to hip ratio.
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Results

Our search strategy and exclusion criteria resulted in a total of 15 trials (17 reports) extracted from 4358 articles that met the objectives and were included in the qualitative and quantitative analysis [ENREF_17_ENREF_17_ENREF_17_ENREF_17_ENREF_17_ENREF_17] [25–41]. The detailed steps of the meta-analysis article selection process are described as a flow diagram in Figure 2. Study duration ranging between 2.5 months and 6 months, published between 1994 and 2012 and enrolling a total of 741 participants. The reported mean age varied between 30.5 and 73.2 years, the corresponding BMI values averaged between 27.8 and 33.8 kg/m². Among the 15 trials adopted for meta-analysis, 14 compared RT vs. AET [26–41], 4 compared CT vs. AET [25,26,32,33,38], and 3 compared CT vs. RT [26,32,33,38]. Four studies reported a dietary co-intervention in the AET and RT groups [27,28,35,40]. General study characteristics are summarized in Table 1.

The pooled estimate of effect size for the effects of RT vs. AET, CT vs. AET, and CT vs. RT on anthropometric and cardiovascular risk factor outcomes are summarized in Table 2.

Pairwise meta-analysis AET vs. RT. The reduction of BW [MD: -1.15 kg (95% CI -2.23 to -0.07), $p=0.04$] ($I^2=34\%$) (Figure S1 in File S1), WC [MD: -1.10 cm (95% CI -1.85 to -0.36), $p=0.004$] ($I^2=0\%$) (Figure S2 in File S1), and FM [MD: -1.14 kg (95% CI -1.83 to -0.45), $p=0.001$] ($I^2=3\%$) (Figure S3

in File S1) was significantly more pronounced in the AET groups as compared to RT, respectively. However, participants in the RT groups showed a significantly more distinct increase in LBM [MD: 1.26 kg (95% CI 0.71 to 1.81), $p<0.00001$] ($I^2=0\%$), when compared to AET protocols (Figure S4 in File S1). Comparison of AET with RT did not result in significantly different outcomes with respect to WHR.

CT vs. AET. CT significantly increased LBM [MD: 0.90 kg (95% CI 0.31 to 1.48), $p=0.003$] ($I^2=0\%$) (Figure S5 in File S1) when compared to the corresponding effects of AET. No other anthropometric parameter was affected in a different fashion by either CT or AET.

CT vs. RT. CT protocols were associated with a significantly more substantial reduction in BW [MD: -2.03 kg (95% CI -2.94 to -1.12), $p<0.0001$] ($I^2=19\%$) (Figure S6 in File S1), WC [MD: -1.57 cm (95% CI -2.38 to -0.75), $p=0.0002$] ($I^2=0\%$) (Figure S7 in File S1), and FM [MD: -1.88 kg (95% CI -2.67 to -1.08), $p<0.00001$] ($I^2=9\%$) (Figure S8 in File S1) when compared to RT strategies, respectively. No significant differences were observed with regard to LBM.

Blood lipids and cardiorespiratory fitness. VO₂max as an indicator of cardiorespiratory fitness was significantly more improved following AET [MD: 2.53 ml/kg/min (95% CI 1.62 to 3.44), $p<0.00001$] ($I^2=0\%$) (Figure S9 in File S1) and CT procedures [MD: 2.79 ml/kg/min (95% CI 1.78 to 3.79), $p<0.00001$] ($I^2=0\%$) (Figure S10 in File S1) when compared to

Table 3. Mean differences estimated from the random effects network meta-analysis model

	BW (kg)	WC (cm)	WHR	FM (kg)	LBM (kg)
AET versus					
RT	−1.34 [−2.28, 0.094]	−1.3 [−2.45, 0.058]	−0.006 [−0.022, 0.011]	−1.00 [−1.90, 0.34]	−1.30 [−3.24, 0.74]
CT versus					
AET	−0.22 [−2.21, 1.11]	−0.22 [−2.09, 1.29]	−0.049 [−0.10, 0.009]	−0.72 [−2.20, 0.469]	0.75 [−2.99, 2.77]
CT versus					
RT	−1.59 [−3.17, 0.058]	−1.54 [−3.32, 0.015]	−0.056 [−0.11, 0.006]	−1.73 [−2.92, −0.30]	−0.53 [−4.36, 1.59]
I ²	0.817 [0.04, 2.44]	0.72 [0.038, 2.66]	0.016 [0.0082, 0.04]	0.49 [0.025, 2.26]	0.86 [0.041, 4.55]
	TC (mg/dl)	HDL-C (mg/dl)	TG (mg/dl)	VO2 max (ml/kg/min)	
AET versus					
RT	−3.82 [−15.49, 6.66]	1.44 [−0.60, 3.38]	−10.8 [−30.22, 8.12]	2.67 [1.47, 3.97]	
CT versus					
AET	10.72 [−15.38, 36.84]	0.86 [−1.64, 3.62]	0.22 [−24.22, 28.98]	−0.019 [−1.74, 1.28]	
CT versus					
RT	6.88 [−20.43, 33.24]	2.30 [−0.54, 5.29]	−10.56 [−37.51, 20.39]	2.66 [1.00, 3.99]	
I ²	5.87 [0.299, 23.88]	0.78 [0.046, 3.26]	9.79 [0.38, 36.97]	0.519 [0.023, 2.24]	

Relative intervention effectiveness is expressed as posterior medians (95% credible intervals); I²: estimated between study heterogeneity standard deviation (95% credible intervals);

AET, aerobic exercise training; BW, body weight; CT, combined training; FM, fat mass; HDL-C, high density lipoprotein cholesterol; LBM, lean body mass; RT, resistance training; TC, total cholesterol; TG, triacylglycerols; VO2 max, maximal oxygen uptake; WC, waist circumference; WHR, waist to hip ratio;

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RT interventions, respectively. No significant differences were observed for TC, LDL-C, HDL-C, and TG, respectively (Table 2).

Network meta-analysis

The pooled estimates of effect size for the comparison of AET vs. RT vs. CT using both direct and indirect evidence on anthropometric and cardiovascular risk factor outcomes are summarized in Table 3 (except LDL-C, since only AET vs. RT trials were available). For each outcome, a common between-study heterogeneity parameter was assumed, to reflect the variability between studies of all interventions (Table 3). The ranking probabilities, and rankings with credibility intervals of AET, RT, and CT for each outcome is presented in Table 4.

Both AET and CT were more effective in reducing body weight compared to RT (Figure S11 in File S1). The network meta-analysis showed that CT was the most powerful exercise intervention to reduce WC (Figure S12 in File S1), and FM (Figure S13 in File S1). Regarding LBM, the observations of the pairwise meta-analysis were also confirmed. No significant differences were observed for WHR, and blood lipids. Maximal oxygen uptake was significantly more pronounced in the AET and CT groups as compared to the RT groups (Figure S14 in File S1).

Due to the structure of the evidence, inconsistency between direct and indirect evidence was only possible for the BW outcome. No evidence of inconsistency was found with Bayesian *p*-values for the difference between direct and indirect evidence all greater than 0.90.

Sensitivity analysis

Sensitivity analysis were performed for obesity, age (≥ 50 years vs. < 50 years) and gender. The primary analysis was confirmed when including only obese subjects. Inclusion of older people (≥ 50 years) resulted in slightly more pronounced effects compared to

younger (< 50 years), while no gender specific differences were observed (data not shown). Furthermore, a meta-analysis of change scores was performed for those trials reporting the corresponding data (10 of 15, see Table S1 in File S1).

Study quality

Three studies were excluded, since study participants were not assigned to intervention groups via randomization [42,43]. Except for one study [34] (semi-randomization) all others were randomized, but only 4 studies reported random sequence generation and only 1 trial reported allocation concealment. None of the studies reported blinding of participants, a lack of which is a common characteristic in exercise interventions (Figure 1). Only one trial performed intention to treat analysis, and appears to have adequate blinding outcome assessment [33]. The retention rate ranged from 63% to 92%, with no significant differences between exercise groups.

Publication Bias

The Begg's and Egger's linear regression tests provided evidence for a potential publication bias for BW ($p = 0.032$) following comparison of AET vs. RT, and for VO₂ max following comparison of CT vs. AET ($p = 0.024$). Funnel plots were generated for outcome measures provided by at least 10 different trials (see Figures S15-S16 in File S1). The plots (with respect to effect size changes for outcome parameters BW (Figure S15 in File S1), and WC (Figure S16 in File S1) in response to training modalities AET vs. RT indicate moderate asymmetry, suggesting that publication bias cannot be completely excluded as a factor of influence on the present meta-analysis. It remains possible that small studies with inconclusive results have not been published or failed to do so.

Table 4. Ranking probabilities of AET, RT, and CT for the different outcome parameters.

	Rank		Probabilities		
	median	95% CrI	best	2 nd best	worst
BW					
AET	2	[1,3]	35.9%	61.4%	2.7%
RT	3	[1,3]	0.6%	4.7%	94.7%
CT	1	[1,3]	63.5%	34%	2.5%
WC					
AET	2	[1,3]	35.8%	62.3%	1.9%
RT	3	[1,3]	0.4%	3.8%	95.7%
CT	1	[1,3]	63.7%	33.9%	2.4%
WHR					
AET	2	[1,3]	3.5%	77.2%	19.2%
RT	3	[1,3]	1.8%	20.7%	77.5%
CT	1	[1,3]	94.7%	2.1%	3.2%
FM					
AET	2	[1,3]	8.7%	86%	5.2%
RT	3	[1,3]	0.7%	5.5%	93.8%
CT	1	[1,3]	90.5%	8.5%	1%
LBM					
AET	3	[1,3]	4.3%	20%	75.6%
RT	1	[1,3]	74.3%	22.5%	3.2%
CT	2	[1,3]	21.3%	57.4%	21.2%
TC					
AET	1	[1,3]	64%	30.5%	5.5%
RT	2	[1,3]	18.1%	56.2%	25.7%
CT	3	[1,3]	17.9%	13.3%	68.8%
HDL-C					
AET	2	[1,3]	22.6%	70.8%	6.6%
RT	3	[1,3]	1.9%	9.1%	89%
CT	1	[1,3]	75.5%	20.1%	4.4%
TG					
AET	2	[1,3]	45.9%	46.6%	7.4%
RT	3	[1,3]	6.4%	21.2%	72.4%
CT	2	[1,3]	47.6%	32.2%	20.2%
VO₂max					
AET	1	[1,3]	51.1%	48.9%	0%
RT	3	[1,3]	0%	0.5%	99.5%
CT	2	[1,3]	48.9%	50.6%	0.5%

AET, aerobic exercise training; BW, body weight; CrI, credible intervals; CT, combined training; FM, fat mass; HDL-C, high density lipoprotein cholesterol; LBM, lean body mass; RT, resistance training; TC, total cholesterol; TG, triacylglycerols; VO₂ max, maximal oxygen uptake; WC, waist circumference; WHR, waist to hip ratio;

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Discussion

To our knowledge, this is the first systematic review investigating the pooled effects of different exercise interventions on anthropometric outcomes, blood lipids and cardiorespiratory fitness. The main findings of this meta-analysis suggest that in subjects with a BMI ≥ 25 kg/m², AET is more efficient in reducing BW, WC and FM as well as in increasing VO₂max

uptake when compared to RT, respectively. However, RT turned out to be more suitable when it comes to an improvement of lean body mass. Furthermore, the present results provide evidence that a combined intervention seems to be the most promising tool for management of overweight and obesity. CT was more powerful in reducing anthropometric risk factors like BW, WC or FM when compared to RT, and more effective in raising LBM when compared to AET. Pooled direct and indirect evidence on these three exercise interventions showed that CT was the most efficacious to reduce anthropometric outcomes such as BW, WC and FM (with the respective ranking probabilities, following Bayesian network meta-analysis: 63%, 63% and 90%).

Since waist circumference correlates with abdominal fat mass and is considered to be an independent predictor of CDV, it can be used as a surrogate marker of abdominal fat mass [44,45]. _ENREF_52 De Koning et al. reported a 2%-increase in CVD risk for each 1cm-gain in WC [45]. By transferring these findings to the results of the present meta-analyses, AET was associated with a reduction in CVD risk that was approximately 2% stronger as in the respective RT counterparts. Moreover, CT resulted in a decline in CVD risk that was by 4% more distinct as compared to the effects of an RT intervention. Aerobic exercise is known to increase the sympathetic tone, and the subsequent release of adrenergic transmitters leads to an increased lipolysis especially in abdominal fat [46].

Regarding lean body mass, the results of the present meta-analyses show that both RT and CT are more effective in raising LBM when compared to AET, respectively. An increase in LBM contributes to the maintenance or may even reflect an increase in resting metabolic rate [47]. Apparently, RT triggers the preservation and buildup of body protein thereby altering the relationship between LBM and FM [48]. In a previous study, it was shown that, if performed twice a week, RT facilitated an increase in LBM by 1–2 kg in the course of 6 months and could prevent age-associated loss of LBM [49].

Results suggest that exercise interventions containing aerobic sessions (whether isolated or as part of a combination training) improve cardiorespiratory fitness when compared to RT as a single training modality. A gain in cardiorespiratory fitness is known to be associated with reduced cardiovascular mortality and cancer incidence in men and women [50,51]. A pooled analysis by Kodama et al. [52] _ENREF_66 investigating the impact of cardiorespiratory fitness on all-cause mortality and cardiovascular events revealed that an increase in VO₂ max in the amount of one metabolic equivalent correlated with a 13%-reduction of all-cause mortality as well as with a 15%-decrease in CHD/CVD risk, respectively. The authors suggested that the +1-MET-improving effects on VO₂ max are comparable to corresponding influences of a decrease in WC (-7 cm), SBP (-5 mmHg), TG (-88 mg/dl), and FG (-18 mg/dl) as well as to increases in HDL-C (+7.72 mg/dl), respectively. When applying these findings to the results of the present meta-analysis, AET outperformed RT as a single training modality with a further 7.5%-risk reduction in all-cause mortality and a further 8.5%-risk reduction in CHD/CVD, respectively.

The present systematic review has several strengths and weaknesses. The meta-analysis was conducted following a stringent protocol, i.e. in all trials, participants were randomly assigned to the intervention groups, and only supervised training protocols were included. Randomized controlled trials are considered to be the gold standard for evaluating the effects of an intervention and are subject to fewer biases as compared to observational studies. The network meta-analysis included all individuals for each outcome. Moreover the present meta-analysis had a substantial sample size (range: 323 to 664) volunteers, thus

providing the power to detect statistically significant mean differences as well as to assess publication bias. Network meta-analysis methods were used to obtain coherent estimates of all treatments relative to each other, using all available evidence and adequately accounting for evidence from 3-arm trials (i.e. avoiding the repeated use of data from such trials in different comparisons). This is of particular importance in this application where there were several trials simultaneously comparing all the interventions. Overall, the estimated between-studies heterogeneity parameters were small for all networks, and there was no evidence of inconsistency, which further strengthens the conclusions. Trial characteristics suggest the consistency/similarity assumption is satisfied, which is confirmed by the statistical analysis.

Limitations of the present review include the limited number of studies and the heterogeneity of the study designs. The trials covered in the meta-analyses showed variations in population characteristics (e.g. overweight, obese, age, number and ratio of male and female participants).

A considerable confounder could be the volume of exercise (min/week) prescribed. Two studies reported exercise intensity in the CT group to be twice as high as compared to their respective RT and/or AET counterparts [25,32,38]. However, a sensitivity analysis excluding these studies confirmed the results of the primary analysis. Other potential confounders included differences in dietary intake and activity performed outside the monitoring and supervision by the investigators. Most studies reported the method of randomization as well as other data required for risk of bias assessment, which might be due to the fact that the trials were performed within the previous 20 years (between 1994 and 2012). However, another major limitation is the size of the study population, i.e. 11 of the 15 trials had a sample size of less than 60 participants, demanding a conservative interpretation of the results. With respect to publication bias, funnel plots for this systematic review showed low to moderate asymmetry suggesting that e.g. lack of published trials with inconclusive results cannot be completely excluded as a confounder of the present meta-analysis (Figure S15-16 in File S1). According to the results of the Begg's and Egger's linear regression tests, there is evidence for a potential publication bias for BW following pairwise comparison of AET vs. RT and VO_2 max following direct comparison of CT vs. AET. Therefore, these results should be interpreted with caution. Future trials should focus on high-quality methodological assessment (allocation concealment, blinding of outcome assessment, and intention-to-treat analysis), long-term effects (≥ 12 months), and larger sample size.

In conclusion, the present systematic review and meta-analysis focused on RCTs mutually comparing AET, RT, and CT. Anthropometrical as well as cardiorespiratory fitness parameters turned out to be significantly more improved following AET or CT protocols as compared to their respective RT counterparts. With respect to the limitations of the present systematic review, a conservative interpretation of the data is required. The primary objective in obesity management is the reduction of body fat. According to the results of the pairwise meta-analysis, reduction of fat mass was significantly more pronounced following AET, and CT as compared to RT. However, addition of RT to AET strategies may prevent loss of LBM, which is a common problem in the course of weight loss in obesity management programs. Evidence from the network meta-analysis suggests that CT is the

most efficacious exercise modality in the prevention and treatment of overweight, and obesity and should therefore be recommended whenever possible.

Supporting Information

File S1 Figure S1: Forest plot showing pooled MD with 95% CI for body weight (kg) for 14 randomized controlled aerobic exercise (AET) vs. resistance (RT) groups. Figure S2: Forest plot showing pooled MD with 95% CI for waist circumference (cm) for 10 randomized controlled aerobic exercise (AET) vs. resistance (RT) groups. Figure S3. Forest plot showing pooled MD with 95% CI for fat mass (kg) for 8 randomized controlled aerobic training vs. resistance training trials. Figure S4: Forest plot showing pooled MD with 95% CI for lean body mass (kg) for 7 randomized controlled aerobic exercise (AET) vs. resistance (RT) groups. Figure S5. Forest plot showing pooled MD with 95% CI for lean body mass (kg) for 3 randomized controlled combined training vs. aerobic training trials. Figure S6. Forest plot showing pooled MD with 95% CI for body weight (kg) for 3 randomized controlled combined training vs. resistance training trials. Figure S7. Forest plot showing pooled MD with 95% CI for waist circumference (cm) for 3 randomized controlled combined training vs. resistance training trials. Figure S8. Forest plot showing pooled MD with 95% CI for fat mass (kg) for 3 randomized controlled combined training vs. resistance training trials. Figure S9. Forest plot showing pooled MD with 95% CI for maximal oxygen uptake (ml/kg/min) for 7 randomized controlled aerobic exercise (AET) vs. resistance (RT) groups. Figure S10. Forest plot showing pooled MD with 95% CI for maximal oxygen uptake (ml/min/kg) for 3 randomized controlled combined training vs. resistance training trials. Figure S11. Scatterplot showing the median with 95% CrI for body weight (kg) for the indirect comparison of different training modalities. Figure S12. Scatterplot showing the median with 95% CrI for waist circumference (cm) for the indirect comparison of different training modalities. Figure S13. Scatterplot showing the median with 95% CrI for fat mass (kg) for the indirect comparison of different training modalities. Figure S14. Scatterplot showing the median with 95% CrI for maximal oxygen uptake (ml/kg/min) for the indirect comparison of different training modalities. Figure S15. Funnel plot showing study precision against the MD effect estimate with 95% CIs for body weight (aerobic vs. resistance training). Figure S16. Funnel plot showing study precision against the MD effect estimate with 95% CIs for waist circumference (aerobic vs. resistance training). Table S1. Pooled estimates (change scores) of effect size (95% confidence intervals) expressed as weighted mean difference for the effects of AET vs. RT, CT vs. AET and CT vs. RT on anthropometric outcomes, blood lipids and cardiorespiratory fitness. Table S2. Baseline study comparability reported as mean and standard deviation. (DOCX)

Author Contributions

Conceived and designed the experiments: LS SD BS GH. Performed the experiments: LS SD BS GH. Analyzed the data: LS SD BS GH. Contributed reagents/materials/analysis tools: LS SD BS GH. Wrote the paper: LS SD BS GH.

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Impact of different training modalities on glycaemic control and blood lipids in patients with type 2 diabetes: a systematic review and network meta-analysis

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Abstract

Aims/hypothesis This study aimed to systematically review randomised controlled trials comparing the effects of aerobic exercise training (AET), resistance training (RT) and combined training (CT) on glycaemic control and blood lipids in patients with type 2 diabetes mellitus.

Methods Searches were performed in MEDLINE, EMBASE and the Cochrane Library. Inclusion criteria were: type 2 diabetes mellitus, adult, supervised training and a minimum intervention period of 8 weeks. Pooled effects were calculated by fixed/random effect pairwise and Bayesian fixed/random effects network meta-analyses.

Results A total of 14 trials enrolling 915 participants were included. AET was more effective than RT in improving HbA_{1c} levels (mean difference [MD] -0.20% [-2.2 mmol/mol]; 95% CI -0.32 , -0.08 ; $p=0.0007$, 10 trials/515 participants) and fasting glucose (MD -0.9 mmol/l; 95% CI -1.71 , -0.09 ; $p=0.03$, 8 trials/245 participants). Compared with AET, CT resulted in a significantly more pronounced reduction in HbA_{1c} (MD -0.17% [-1.87 mmol/mol]; 95% CI -0.31 , -0.03 ; $p=0.02$, 9 trials/493 participants). Compared with RT, the MD of the change in HbA_{1c} (MD -0.62% , [-6.82 mmol/mol]; 95% CI -0.95 ,

-0.30 ; $p=0.0002$, 5 trials/362 participants], fasting glucose (MD -1.99 mmol/l; 95% CI -3.07 , -0.90 ; $p=0.0003$, 3 trials/99 participants) and triacylglycerols (MD -0.28 mmol/l; 95% CI -0.46 , -0.10 ; $p=0.003$, 4 trials/213 participants) were all in favour of CT. The exclusion of trials with a high risk of bias yielded only non-significant results.

Conclusions/interpretation The present data suggest that CT might be the most efficacious exercise modality to improve glycaemic control and blood lipids. Interpretation with respect to clinical relevance is limited by the low quality of the studies included and the limited information on the clinically important outcomes or adverse effects of exercise.

Keywords Aerobic exercise · Combined training · Network meta-analysis · Resistance training · Systematic review

Abbreviations

AET	Aerobic exercise training
BW	Body weight
CT	Combined training
DBP	Diastolic blood pressure
FG	Fasting glucose
MD	Mean difference
RT	Resistance training
SBP	Systolic blood pressure
TC	Total cholesterol
TG	Triacylglycerols

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Introduction

Increased physical activity and improved nutritional habits in the form of hypocaloric diets (of varying macronutrient compositions) are of particular importance to decelerate the manifestations of type 2 diabetes [1–3]. The ADA and the

American College of Sports Medicine have stated that a combination of resistance training (RT) and aerobic exercise training (AET) of at least 150 min of moderate-intensity exercise per week may be more effective in improving glycaemic control than focusing solely on one single training modality (evidence category B) [4].

The isolated effects of either RT or AET, or a combination of both (combined training [CT]), on anthropometric, cardiac and metabolic risk factors have been meta-analysed by Snowling and Hopkins [5] as well as by Chudyk and Petrella [6]. Both studies reported that the reduction in HbA_{1c} and fasting glucose (FG) levels, systolic blood pressure (SBP), waist circumference, HDL and triacylglycerols (TG) was more pronounced following AET and CT compared with RT. In addition, HbA_{1c}- and blood pressure-lowering effects of RT were shown. However, all these systematic reviews included trials in which training modalities were compared with the data from a sedentary control group [7, 8].

To date, no systematic review has compared the direct and indirect effects of these three different training modalities on the outcomes of glycaemic control and blood lipids in patients with type 2 diabetes. A recent pairwise meta-analysis comparing RT (all supervised) with AET (not all supervised) exercise in patients with type 2 diabetes concluded that although differences in some outcome variables reached statistical significance, there was no evidence that they were of clinical relevance [9]. In a recently published network meta-analysis, we were able to demonstrate that CT is ranked as the most likely effective exercise model in the treatment of overweight and obesity [10].

The aim of the present study was to assess the efficacy of AET, RT and CT on glycaemic control, blood pressure and blood lipids in patients with type 2 diabetes mellitus in a systematic review including a pairwise and network meta-analysis of randomised trials. The importance of supervised training has been demonstrated by Umpierre et al, who showed that structured training compared with training advice only significantly improved glycaemic control in patients with type 2 diabetes [11]. Based upon these findings, only trials that conducted an exercise intervention which was guided by supervised training were enrolled in the present systematic review and meta-analyses.

Methods

The review was registered in PROSPERO International Prospective Register of Systematic Reviews (www.crd.york.ac.uk/prospero/index.asp, identifier CRD42014007502). However, no study protocol was published before the initiation of the meta-analysis.

Literature search

Queries of the literature were performed using the electronic databases MEDLINE (until 2 May 2014), EMBASE (until 2 May 2014) and the Cochrane Central Register of Controlled Trials (until 2 May 2014) with no restrictions. The following keywords were used: ('strength' OR 'resistance' OR 'aerobic' OR 'endurance' OR 'combined training' OR 'progressive' OR 'walking' OR 'interval training' OR 'weight lifting') AND ('training' OR 'exercise' OR 'physical activity') AND ('diabetes' OR 'glycemic' OR 'glycaemia' OR 'glycaemic' OR 'glycemia' OR 'HbA_{1c}' OR 'A_{1c}' OR 'glycated' OR 'glycosylated' OR 'glucose' OR 'lipids' OR 'body weight' OR 'blood pressure') AND ('randomized controlled trial' OR 'randomized' OR 'clinical trials as topic' OR 'placebo' OR 'randomly' OR 'trial') NOT ('animals' NOT 'humans').

Moreover, the reference lists from the retrieved articles, systematic reviews and meta-analyses were checked to search for further relevant studies. This systematic review was planned, conducted and reported in adherence with standards of quality for reporting meta-analyses [12]. The entire literature search was conducted independently by two authors (L. Schwingshackl and B. Missbach), with disagreements resolved by consensus. The detailed search strategy for MEDLINE is given in the electronic supplementary material (ESM [Methods](#)).

Eligibility criteria

Studies were included in the meta-analysis if they met all of the following criteria: (1) a randomised controlled design; (2) a minimum intervention period of 8 weeks; (3) patients with type 2 diabetes; (4) patients' age ≥ 19 years; (5) a comparison of either AET vs RT and/or CT vs AET and/or CT vs RT; (6) an assessment of at least one of the following outcome markers: HbA_{1c}, blood glucose, body weight (BW), blood pressure or blood lipids (total cholesterol [TC], LDL, HDL and TG); (7) the reporting of changes from baseline value scores with SDs (or data suitable to calculate these variables: SE and 95% CI); if the SDs of the changes from baseline value scores were not available, post-intervention values were imputed, according to the *Cochrane Handbook* [13]; (8) training that was conducted under direct (guided by a physiotherapist in training classes, hospital gyms, etc.) or partial supervision, and was not home-based; and (9) the exclusion of studies with a dietary co-intervention that was not applied in all the intervention groups. All abstracts and full texts were independently assessed for eligibility by two authors (L. Schwingshackl and B. Missbach).

Risk of bias assessment

Full copies of the studies were independently assessed by two authors (L. Schwingshackl and B. Missbach) for

methodological quality using the risk of bias assessment tool from the Cochrane Collaboration [13, 14]. The following sources of bias were detected: selection bias (random sequence generation and allocation concealment), detection bias (blinding of outcome assessment), blinding of participants and personnel (performance bias), attrition bias (incomplete outcome data) and reporting bias (selective reporting) (ESM Fig. 1).

Data extraction and statistical analysis

The following data were extracted from each study: the first author's last name, publication year, study duration, participant's sex, age and BMI, sample size, duration of diabetes, HbA_{1c} at baseline, drug treatment, change of treatment during the trial, treatment effects, intervention type, dose, intensity and frequency, and differences in the means of two time points or post-intervention mean values with corresponding SDs. For each outcome measure of interest, pairwise and network random effects meta-analyses were performed in order to determine the pooled relative effect of each intervention relative to every other intervention in terms of the mean differences (MDs) between the changes from baseline value scores (or post-intervention values) of the different interventions. To process the data for the meta-analysis, we imputed the data for the changes from baseline means and their SDs. When the SDs for the changes from baseline values were not available [15–20], the post-intervention values with the corresponding SDs were imputed, according to the guidelines of the *Cochrane Handbook* [13].

Data were pooled if outcomes were reported by at least three studies. Heterogeneity between trial results was tested with a Cochran's Q test. A value for I^2 of >50% was considered to represent substantial heterogeneity [21]. When substantial heterogeneity was present, the random effects model was used to estimate MDs with 95% CIs. Forest plots were generated to illustrate the study-specific effect sizes along with a 95% CI. To determine the presence of publication bias, the symmetry of the funnel plots in which mean MDs were plotted against their corresponding SEs were assessed. Additionally, Begg's and Egger's regression tests were performed to detect small study effects [22, 23].

Separate pairwise meta-analyses were first used to compare all the interventions. Network meta-analysis was then used to synthesise all the available evidence [24]. Network meta-analysis methods are extensions of the standard pairwise meta-analysis model that enable a simultaneous comparison of multiple interventions while preserving the internal randomisation of individual trials. They have the advantage of adequately accounting for the correlation in relative effect estimates from three-arm trials as well as providing a single coherent summary of all the evidence. Random effects network meta-analysis models were used when substantial heterogeneity was found in any of the pairwise comparisons for that outcome. Otherwise, the choice between fixed and

random effects was made by comparing the deviance information criteria for each model [24, 25]. The model with the lowest deviance information criterion was chosen (differences >3 are considered meaningful). Pooled effect sizes from the network meta-analyses are presented as posterior medians and 95% credible intervals (i.e. the Bayesian equivalent of CIs) in the appropriate units, along with the estimated between-study heterogeneity.

For pairwise meta-analyses, data were analysed using Review Manager 5.1 software, provided by the Cochrane Collaboration (<http://ims.Cochrane.org/revman>). Network meta-analyses were conducted using Markov chain Monte Carlo simulation implemented with the open-source software WinBUGS, version 1.4.3 [26]. The WinBUGS code used is freely available online [24, 27] (program 'TSD2-5aRE_Normal_id.odc' or 'TSD2-5aFE_Normal_id.odc').

Minimally informative normal priors were used for all treatment effect variables and a uniform prior (0, 150) was used for the between-study SD (heterogeneity) variable. Sensitivity to this prior was assessed, but there was no meaningful change in the relative effects or overall conclusions.

Three Markov chain Monte Carlo chains were used to assess convergence using Brooks–Gelman–Rubin plots and inspection of the trace plots [28]. Convergence was achieved after 20,000 iterations for all outcomes. Posterior summaries were then obtained from a further simulation of 50,000 iterations in each of the three chains (giving 150,000 in total), resulting in a small Monte Carlo error.

The potential for inconsistency was assessed by inspection of the available evidence. In case of possible inconsistency, Bayesian p values for the difference between direct and indirect evidence were calculated, and direct and indirect estimates were compared [29, 30].

Results

Overall, a total of 14 trials (16 reports) extracted from 9,477 articles met the eligibility requirements and were included for the present systematic review and meta-analysis [15–20, 31–40]. One study was excluded since it was not described as randomised [41], and two trials provided no information on whether the AET was supervised [42, 43]. The detailed steps of the article selection process for the meta-analysis are described as a flow diagram in ESM Fig. 2. The studies were published between 2003 and 2013 and had enrolled a total of 915 participants. The study duration ranged between 2 and 12 months; the patients' mean age was between 49 and 62.5 years, and their BMI between 27.1 and 43.8 kg/m². Fourteen trials met the objectives for meta-analysis: 10 compared RT vs AET, 9 compared CT vs AET, and 5 compared CT vs RT (ESM Fig. 3). The general and specific study

characteristics are summarised in Table 1, ESM Table 1 and ESM Table 2.

The pairwise pooled estimate of effect size for the effects of RT vs AET, CT vs AET and CT vs RT on glycaemic control, blood pressure and blood lipids are summarised in Table 2.

Pairwise meta-analysis

Primary outcome The reduction in HbA_{1c} (MD −0.20% [−2.2 mmol/mol]; 95% CI −0.32, −0.08; $p=0.0007$, $I^2=26\%$, 10 trials, 515 participants) (ESM Fig. 4) was

significantly more pronounced in the AET groups compared with the RT groups. When compared with AET and RT, the CT protocols resulted in a significant reduction in HbA_{1c} (MD −0.17% [−1.87 mmol/mol]; 95% CI −0.31 to −0.03; $p=0.02$, $I^2=21\%$, 9 trials, 493 participants) (ESM Fig. 5) and (MD −0.62%, [−6.82 mmol/mol]; 95% CI −0.95, −0.30; $p=0.0002$, $I^2=74\%$, 5 trials, 362 participants) (ESM Fig. 6).

Secondary outcomes No significant differences were observed for BW, diastolic blood pressure (DBP), SBP, TC,

Table 1 General study characteristics

Reference	Sample size, mean baseline BMI (kg/m ²)	Mean age (years), female (%)	Study duration (months)	Comparisons	Timing of post-intervention measurement since last exercise session	Findings
Bacchi et al 2012 [31]	40 29.35	56.4 30	4	RT vs AET	n.d.	RT: / AET: ↑↑ $\dot{V}O_{2max}$
Balducci et al 2010 [17]	42 30	62.5 38	12	AET vs CT	n.d.	AET: ↓ WC; ↑ $\dot{V}O_{2max}$, HDL CT: ↓ BW, WC; ↑ $\dot{V}O_{2max}$, HDL
Church et al 2010 [32]	221 34.9	55.3 62	9	RT vs AET vs CT	48–72 h after last exercise test	RT: ↓ FM AET: ↓ LBM CT: ↓ BW, FM, ↑ $\dot{V}O_{2max}$
Cuff et al 2003 [33]	19 32.9	61.4 100	4	AET vs CT	n.d.	AET: ↓↓ BW CT: ↓↓ BW
Gram et al 2010 [19]	68 32.3	60.6 46	4	AET vs CT	n.d.	AET: / CT: LDL ↓↓
Jorge et al 2011 [15] de Oliveira et al 2012 [20]	36 30.6	54.7 61	3	RT vs AET vs CT	n.d.	RT: ↓ TC, HDL, TG AET: ↓ TC, HDL, TG, ↑ $\dot{V}O_{2max}$ CT: ↓ TC, TG
Kwon et al 2011 [34] Ku et al 2010 [35]	28 27.1	55.9 100	3	RT vs AET	n.d.	RT: ↓ BW AET: ↓ BW, ↑ $\dot{V}O_{2max}$
Kadoglou et al 2013 [36]	66 32.1	57.4 27	6	RT vs AET vs CT	n.d.	AET: ↓ SBP, FG, HbA _{1c} , HOMA-IR, FI, TC, TG; ↑ $\dot{V}O_{2max}$ RT: ↓ SBP, FG, HbA _{1c} , TC, TG, HOMR-IR, FI CT: ↓ SBP, FG, HbA _{1c} , HOMA-IR, FI, TC, TG, FM ↑ $\dot{V}O_{2max}$
Lambers et al 2008 [18]	35 29.8	54 34	3	AET vs CT	After last exercise and overnight fasting	AET: / CT: ↓↓ HbA _{1c} , ↓↓ TC
Moe et al 2011 [37]	26 30	57 0	3	RT vs AET	48 h after last exercise tests	AET: ↓ HbA _{1c} ; ↑ $\dot{V}O_{2max}$ RT: ↓ WHR, HbA _{1c}
Ng et al 2010 [38]	60 27.6	58 68	2	RT vs AET	n.d.	RT: ↓↓ WC AET: ↑↑ $\dot{V}O_{2max}$
Sigal et al 2007 [16]	188 34.9	54 63	6	RT vs AET vs CT	n.d.	RT: ↓↓ HbA _{1c} AET: ↓↓ HbA _{1c} , BW, WC, FM CT: ↓↓ HbA _{1c}
Sukala et al 2012 [39]	26 43.8	49 72	4	RT vs AET	72 h after last exercise tests	RT: / AET: ↓ TG
Yavari et al 2012 [40]	60 29.5	50.2 n.d. n.d.	12	RT vs AET vs CT	n.d.	AET: ↓ HbA _{1c} , FG, TG ↑ $\dot{V}O_{2max}$ RT: ↓ HbA _{1c} , FG, FM, ↑ $\dot{V}O_{2max}$ CT: ↓ HbA _{1c} , FG, BMI, FM ↑ $\dot{V}O_{2max}$

/, no significant within/between-group changes; ↓↓, ↑↑ significant between-group changes (decreases and increases, respectively; including control group comparison); ↓, ↑ significant within-group changes (decreases and increases, respectively)

n.d., no data; $\dot{V}O_{2max}$, maximal oxygen uptake; WC, waist circumference; FM, fat mass; LBM, lean body mass; FI, fasting insulin

Table 2 Pooled estimates (pairwise fixed or random effect meta-analysis) of effect size (95% CIs) expressed as MD for the effects of AET vs RT, CT vs AET and CT vs RT on glycaemic control, blood lipids, blood pressure and BW

Outcomes	No. of studies	Sample size	MD ^a	95% CI	<i>p</i> values	Inconsistency <i>I</i> ²	Egger test
AET vs RT							
HbA _{1c} (%)	10	515	−0.20	−0.32, −0.08	0.0007	26%	0.80
HbA _{1c} (mmol/mol)			−2.20	−3.52, −0.88			
FG (mmol/l)	8	245	−0.90	−1.71, −0.09	0.03	72%	0.66
TC (mmol/l)	8	262	0.06	−0.11, 0.22	0.50	26%	0.64
LDL (mmol/l)	9	372	−0.03	−0.15, 0.09	0.62	32%	0.28
HDL (mmol/l)	9	367	0.04	−0.05, 0.13	0.35	81%	0.61
TG (mmol/l)	9	367	0.02	−0.11, 0.15	0.77	40%	0.56
DBP (mmHg)	8	342	1.03	−0.86, 2.92	0.29	38%	0.02
SBP (mmHg)	8	342	−2.97	−7.72, 1.79	0.22	60%	0.64
BW (kg)	7	410	−0.32	−0.78, 0.14	0.17	2%	0.65
CT vs AET							
HbA _{1c} (%)	9	493	−0.17	−0.31, −0.03	0.02	21%	0.17
HbA _{1c} (mmol/mol)			−1.87	−3.41, −0.33			
FG (mmol/l)	4	132	−0.59	−1.25, 0.08	0.08	49%	0.87
TC (mmol/l)	6	219	−0.15	−0.35, 0.05	0.15	5%	0.13
LDL (mmol/l)	6	291	−0.06	−0.24, 0.13	0.54	5%	0.50
HDL (mmol/l)	7	326	0.03	−0.03, 0.09	0.31	0%	0.94
TG (mmol/l)	6	281	−0.16	−0.35, 0.03	0.10	0%	0.42
DBP (mmHg)	6	291	−1.83	−4.67, 1.01	0.21	51%	0.57
SBP (mmHg)	6	291	−0.81	−4.22, 2.61	0.64	0%	0.09
BW (kg)	8	450	−0.95	−1.93, 0.02	0.05	42%	0.97
CT vs RT							
HbA _{1c} (%)	5	362	−0.62	−0.95, −0.30	0.0002	74%	0.69
HbA _{1c} (mmol/mol)			−6.82	−10.45, −3.3			
FG (mmol/l)	3	99	−1.99	−3.07, −0.90	0.0003	61%	0.45
TC (mmol/l)	3	99	−0.12	−0.90, 0.65	0.75	84%	0.72
LDL (mmol/l)	4	218	−0.14	−0.68, 0.39	0.59	83%	0.74
HDL (mmol/l)	4	218	0.10	−0.11, 0.32	0.33	91%	0.48
TG (mmol/l)	4	213	−0.28	−0.46, −0.10	0.003	0%	0.31
DBP (mmHg)	4	213	−1.13	−3.55, 1.29	0.36	0%	0.50
SBP (mmHg)	4	206	−4.42	−8.62, −0.21	0.04	41%	0.08
BW (kg)	4	317	−1.04	−2.07, −0.00	0.05	0%	0.03

^a Fixed effect meta-analysis if *I*² ≤50%

LDL, HDL and TG between AET and RT. However, AET resulted in a significant reduction in FG (MD −0.90 mmol/l; 95% CI −1.71, −0.09; *p*=0.03, *I*²=72%, 8 trials, 245 participants) compared with RT (ESM Fig. 7). Compared with RT, CT resulted in a more pronounced decrease in FG (MD −1.99 mmol/l; 95% CI −3.07, −0.90; *p*=0.0003, *I*²=61%, 3 trials, 99 participants) (ESM Fig. 8), TG (MD −0.28 mmol/l; 95% CI −0.46, −0.10; *p*=0.003, *I*²=0%, 4 trials, 213 participants) (ESM Fig. 9) and SBP (MD −4.42 mmHg; 95% CI −8.62, −0.21; *p*=0.04, *I*²=41%, 4 trials, 206 participants) (ESM Fig. 10).

Network meta-analysis

ESM Fig. 3 shows the network of the included trials. The pooled estimates of effect size for the comparison of AET vs RT vs CT using both direct and indirect evidence on glycaemic control and cardiovascular risk outcomes are summarised in ESM Table 3 (a fixed effect network meta-analysis for BW and TG, since *I*² ≤50%). For each outcome, a common between-study heterogeneity variable was assumed to reflect the variability between studies of all the interventions (ESM Table 3). The ranking

probabilities of AET, RT and CT for each outcome are presented in ESM Table 4.

Both AET and CT were significantly more effective in reducing HbA_{1c} when compared with RT. As shown in ESM Table 4, CT turned out to be the most effective exercise intervention with respect to reducing HbA_{1c}, FG, TC, LDL, TG, DBP, SBP and BW, and increasing HDL. CT resulted in a high (>75%) probability of being best for most outcomes. There is greater uncertainty regarding which treatment is the best for LDL- and TC, although again CT yielded the highest probability of being best.

No evidence of inconsistency was found with Bayesian *p* values for the difference between direct and indirect evidence all greater than 0.90.

Risk of bias

The dropout rates ranged from 0% to 31%, with five studies reporting dropout rates <10% (ESM Table 1). Seven trials reported random sequence generation [16, 31, 32, 36–39], and only five trials reported allocation concealment [16, 31, 32, 37, 38]. None of the studies reported the blinding of volunteers towards the mode of intervention (ESM Fig. 1). Eight trials performed intention-to-treat analysis [15–17, 32, 35, 37–39], and six trials appear to have had adequate blinding of the outcome assessment [16, 17, 31, 32, 37, 38]. High risk of bias was defined as fewer than four out of a maximum yield of six low risk of bias items using the risk of bias assessment tool from the Cochrane Collaboration (ESM Fig. 1). Seven high risk of bias trials (nine reports) were identified [15, 19,

20, 33–36, 39, 40], and sensitivity analyses were performed for studies with a high vs low risk of bias.

Subgroup analysis/sensitivity analysis

Subgroup analyses were performed comparing short-term (<6 months) vs long-term (≥6 months) trials (ESM Figs 11–13), obese (BMI ≥30 kg/m²) vs non-obese (BMI <30 kg/m²) participants (ESM Figs 14–16) and sample size (≥50 vs <50) (ESM Fig. 17–19). Overall, pooling the long-term trials resulted in significantly greater reductions of HbA_{1c} compared with short-term trials for all comparison groups. Furthermore, including only obese patients resulted in significant reductions in HbA_{1c}. A smaller vs bigger sample size showed non-significant differences for HbA_{1c} when comparing AET vs RT. In contrast, comparisons for CT yielded significantly higher reductions in trials with a bigger sample size when compared with either AET or RT. Subgroup analysis comparing different measurement time points for HbA_{1c} provided no additional information (ESM Figs 20–22). Sensitivity analyses excluding trials with a high risk of bias changed the summary estimates and became statistically non-significant (Table 3).

Publication bias

Begg's and Egger's regression tests provided no evidence of a substantial publication bias. Funnel plots were generated only if specific outcome measures were provided by at least ten different trials. The plot with respect to change in effect size

Table 3 Low risk of bias sensitivity analysis (pairwise fixed/random effect meta-analysis) of effect size (95% CIs) expressed as weighted MD for the effects of AET vs RT, CT vs AET and CT vs RT on glycaemic control, blood lipids, blood pressure and BW

Outcomes	No. of studies	Sample size	MD	95% CI	<i>p</i> values	Inconsistency <i>I</i> ²
AET vs RT						
HbA _{1c} (%)	5	371	−0.07	−0.22, 0.08	0.38	0%
HbA _{1c} (mmol/mol)			−0.77	−2.42, −0.88		
FG (mmol/l)	3	101	−0.12	−0.68, 0.44	0.68	0%
TC (mmol/l)	3	121	0.11	−0.12, 0.33	0.35	0%
LDL (mmol/l)	4	226	−0.06	−0.20, 0.09	0.34	0%
HDL (mmol/l)	4	226	0.00	−0.09, 0.09	0.96	68%
TG (mmol/l)	4	226	0.08	−0.09, 0.26	0.70	0%
DBP (mmHg)	4	226	−0.86	−3.62, 1.89	0.54	0%
SBP (mmHg)	4	226	−1.18	−7.53, 5.17	0.72	60%
BW (kg)	3	310	−0.26	−0.82, 0.29	0.36	0%
CT vs AET						
HbA _{1c} (%)	4	332	−0.10	−0.32, 0.11	0.35	0%
HbA _{1c} (mmol/mol)			−1.1	−3.52, −1.21		
HDL (mmol/l)	3	184	0.07	−0.02, 0.15	0.12	34%
TG (mmol/l)	3	184	−0.28	−0.62, 0.07	0.11	19%
BW (kg)	4	332	−1.80	−7.86, 4.27	0.56	64%

for HbA_{1c} in response to AET vs RT indicates little asymmetry. Thus, publication bias cannot be completely excluded as a factor affecting the results of the present meta-analysis (ESM Fig. 23).

Discussion

According to our literature search, this is the first network meta-analysis comparing the pooled effects of AET, RT and CT on glycaemic control, blood pressure and blood lipids in patients with type 2 diabetes. The results of the present meta-analyses showed that, in patients with established diabetes, AET might be more effective in reducing HbA_{1c} and FG when compared with RT. CT was more powerful in reducing HbA_{1c} compared with AET, and more effective in reducing HbA_{1c}, FG and TG when compared with RT. However, these results could not be confirmed when only low risk of bias studies were included. Pooling both direct and indirect evidence on AET, RT and CT via network meta-analysis demonstrated that CT was the most efficacious exercise intervention regarding its impact on HbA_{1c}, FG, HDL, TG, DBP and BW (with the respective probabilities of being ranked best following Bayesian network meta-analysis of 94%, 94%, 78%, 99%, 84% and 97%).

HbA_{1c} is not unanimously regarded to be a valid predictor of cardiovascular disease, thereby limiting the relevance of the present findings with respect to their clinical implications. The interpretation of the present data is further restricted by the fact that none of the studies evaluated the impact of their interventions on clinical outcomes. Data from epidemiological studies suggest that greater physical activity is associated with a reduced risk of all-cause mortality, mortality from cardiovascular disease and risk of type 2 diabetes [44–47].

A recent meta-analysis comparing RT with AET concluded that there is no evidence that RT differs from AET in its impact on cardiovascular risk factors and safety [9]. However, for some risk factors, the ranking probabilities of the Bayesian network meta-analysis suggest that AET was the second best exercise modality. However, these probabilities should not be overinterpreted, particularly since they are not very large (not close to 80–90%). A dose–response meta-regression analysis by Umpierre et al [48] summarised the effects of CT, AET and RT on glycaemic control in patients with type 2 diabetes and concluded that the reduction in HbA_{1c} was associated with exercise frequency in supervised AET, and with the weekly volume of RT in supervised CT. Regarding the optimal dose, the authors speculated that there should be a minimal amount of AET (33 min per session) to elicit the effects of high-volume RT in CT [11].

The results of this meta-analysis are in line with data published by Chudyk et al [6] comparing CT, AET and RT

with control groups. The authors concluded that RT is not significantly related to changes in HbA_{1c} levels or to changes in SBP in patients with type 2 diabetes if it is not combined with other forms of exercise. Moreover, splitting AET and RT sessions between different days might have additional benefits for glycaemic control [49]. This indicates that more elaborated training programmes might be of relevance.

According to a mechanistic model linking the combination of AET and RT with the improvement in glycaemic control in type 2 diabetes, RT enhances insulin sensitivity [50] via an increase in glucose transporter (GLUT)-4 content and an amplification of insulin signalling in muscle [51]. Similarly, AET increased GLUT-4 expression in the adipose tissue and skeletal muscle of patients with type 2 diabetes; however, the benefit of this adaption appears to be dependent on optimal beta cell function [52].

One strength of this systematic review is the application of a network meta-analysis. Direct and indirect evidence was used, taking into account the fact that AET, RT and CT were compared simultaneously in some studies. However, the multiple use of data from three-arm trials will lead to an overestimation of the corresponding data and should be avoided. The relevance of the present data is further emphasised by the small estimated between-studies heterogeneity variables as well as by the fact that there was no evidence of inconsistencies.

On the other hand, this systematic review has several limitations that should be taken into account when interpreting its findings. There is evidence that supervised exercise is more effective than unsupervised training [11], but in practice it seems unlikely that most patients would have access to supervised exercise regimens of this intensity. It is possible that either AET, RT or CT may be easier to perform effectively without supervision, thus affecting the external validity of these results since only studies with supervised training were included.

Although the network meta-analysis included all individuals for each outcome, the sample size of volunteers might be considered low when compared with drug trials. Several potential risk of bias characteristics were identified in the 14 included trials (7 trials described random sequence generation, 5 trials performed allocation concealment, 8 trials performed intention-to-treat analysis, and 6 trials had adequate blinding of outcome assessment). Taken together, more than 50% of the included trials were judged as being at high risk of bias. Therefore, the results of the present meta-analyses should be interpreted in a conservative manner.

There were some heterogeneities in study design especially with respect to the population characteristics (e.g. duration of type 2 diabetes, study length, BMI, age and ratio of male to female participants). Subgroup analyses showed that long-term trials as well as trials including obese participants with type 2 diabetes resulted in more pronounced beneficial effects

on HbA_{1c}, which might be due to carrying forward HbA_{1c} values under conditions of high dropout rates. However, no significant differences could be observed following a comparison of the dropout rates between short- and long-term studies in the present network meta-analysis. Another confounder might be the variations in the volume of exercise (min per week) prescribed. One study reported exercise duration (min per session) in the CT group to be twice as high as with their respective RT and/or AET counterparts [16]. However, a sensitivity analysis excluding this trial was able to confirm the results of the primary analysis.

With respect to the potential side effects (ESM Table 1), eight trials in the present meta-analysis reported adverse events such as hypoglycaemia, back pain, shoulder pain, musculoskeletal injury, tendonitis and other musculoskeletal discomforts following exercise, with no significant differences between the intervention groups. However, it remains possible that the number of adverse events will increase with the duration and intensity of exercise.

This systematic review and meta-analysis focused on randomised controlled trials comparing AET, RT and CT. Compared with AET or RT, CT interventions resulted in significantly more pronounced improvements in variables related to glycaemic control. With respect to single types of exercise intervention, AET was more effective in reducing HbA_{1c} and FG when compared with RT. However, the interpretation of these findings with respect to their clinical relevance is limited by the overall low to moderate quality of the studies included, the lack of information on clinically important outcomes, and the limited information on the adverse effects of exercise.

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Contribution statement LS and GH conceived this study, LS, BM and SD analysed the data, and LS, BM, JK and GH contributed to the collection of data. All authors critically reviewed various drafts of the manuscript, and all authors approved the final version. GH is responsible for the integrity of the work as a whole.

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RESEARCH

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Impact of long-term lifestyle programmes on weight loss and cardiovascular risk factors in overweight/obese participants: a systematic review and network meta-analysis

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Abstract

Background: The aim of this meta-analysis was to compare the long-term efficacy of diet plus exercise (D + E) vs. diet (D), D + E vs. exercise (E) and D vs. E on anthropometric outcomes and cardiovascular risk factors in overweight and obese participants.

Methods: Electronic searches were performed in MEDLINE and the Cochrane Central Register of controlled trials. Inclusion criteria were as follows: body mass index ≥ 25 kg/m² and a minimum intervention period including follow-up of ≥ 12 months. Outcomes of interest were as follows: anthropometric parameters, blood lipids, blood pressure and cardiorespiratory fitness. Pooled effects were calculated using pairwise random effects and Bayesian random effects network meta-analysis. Results of the corresponding fixed effects models were compared in sensitivity analyses.

Results: Overall, 22 trials (24 reports) met the inclusion criteria and 21 (including 3,521 participants) of them were included in the quantitative analysis. As compared with D, D + E resulted in a significantly more pronounced reduction in body weight [mean differences (MD): -1.38 kg, 95% confidence interval (CI) -1.98 to -0.79], and fat mass (MD: -1.65 kg, 95% CI -2.81 to -0.49), respectively. When comparing D + E with E, MD in change of body weight (-4.13 kg, 95% CI -5.62 to -2.64), waist circumference (-3.00 cm, 95% CI -5.81 to -0.20), and fat mass (-3.60 kg, 95% CI -6.15 to -1.05) was in favour of combined diet and exercise, respectively. Comparing E vs. D, diet resulted in a significantly more pronounced decrease in body weight (MD: -2.93 kg, 95% CI -4.18 to -1.68), and fat mass (MD: -2.20 kg, 95% CI -3.75 to -0.66). D + E yielded also the greatest reductions with respect to blood lipids and blood pressure when compared to single applications of D and E, respectively. Results from the network meta-analyses confirmed these findings.

Conclusions: Moderate-quality evidence from the present network meta-analysis suggests that D + E can be highly recommended for long-term obesity management. Furthermore, the evidence suggests a moderate superiority of D over E with respect to anthropometric outcomes.

Systematic review registration: PROSPERO CRD42013003906

Keywords: Lifestyle, Obesity, Network meta-analysis, Systematic review, Diet, Exercise

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Background

In 2008, an estimated 1.4 billion adults were overweight meaning that the prevalence of obesity has more than doubled since 1980. Of these, over 200 million men and nearly 300 million women were obese [1]. Overweight (body mass index (BMI): ≥ 25 kg/m²) and obesity (BMI: ≥ 30 kg/m²) are independent risk factors for non-communicable diseases, especially cardiovascular diseases (CVD) and several types of cancer [2,3]. Exercise and diet are cornerstones in the prevention and management of overweight and obesity. Reductions of fat mass, primarily visceral adipose tissue, are major objectives. Energy expenditure increases with physical activity, especially with aerobic exercise or combined aerobic and resistance training [4,5]. Caloric restriction induces weight loss by negative energy balance. Evidence from meta-analyses indicates that low-carbohydrate diets have slightly more favourable effects on body weight as compared to low-fat diets. Nevertheless, independent of macronutrient composition, the long-term health effects of diets are as yet unknown, and the observed outcomes appear of little clinical significance [6-9]. Regarding exercise training, results from recent network meta-analyses indicate that combined aerobic and resistance exercise is the most effective training modality in the treatment/prevention of overweight/obesity and type 2 diabetes mellitus [4,10].

Previous meta-analyses by Shaw et al. [11] (including trials: ≥ 3 months length) as well as Wu et al. [12] (≥ 6 months) focused on intervention trials comparing diet plus exercise (D + E) vs. diet (D) on body weight and BMI as outcome parameters, but anthropometric outcomes such as waist circumference, fat mass, waist to hip ratio and cardiovascular risk factors (blood lipids, blood pressure and cardiorespiratory fitness) were not included. To the best of our knowledge, to date, no meta-analysis has compared the head-to-head and indirect long-term (≥ 12 months) effects of D + E vs. D vs. E on anthropometric parameters and cardiovascular risk factors. Therefore, the aim of this study was to conduct a systematic review with pairwise and network meta-analysis of randomized controlled trials to combine the direct and indirect evidence on the efficacy of different lifestyle long-term weight-reducing interventions on anthropometric parameters, blood lipids, blood pressure and cardiorespiratory fitness in participants with a BMI ≥ 25 kg/m².

Methods

The review protocol has been registered in PROSPERO International Prospective Register of Systematic Reviews (crd.york.ac.uk/prospero/index.asp Identifier: CRD42013003906).

Literature search

Queries of literature were performed using the electronic databases MEDLINE (between 1966 and June 2014) and

the Cochrane Trial Register (until June 2014) with no restrictions to language and calendar date using the following search terms: ("lifestyle" OR "exercise" OR "diet") AND ("body weight" OR "lipids") AND ("randomized controlled trial" OR "randomized" OR "clinical trials as topic" OR "placebo" OR "randomly" OR "trial") NOT ("animals" NOT "humans"). Moreover, the reference lists from retrieved articles and systematic reviews and meta-analyses were checked to search for further relevant studies. This systematic review was planned, conducted and reported in adherence to standards of quality for reporting meta-analyses [13]. Literature search was conducted independently by two authors (LS, GH), with disagreements resolved by consensus.

Eligibility criteria

Studies were included in the meta-analysis if they met all of the following criteria: (i) randomized controlled design; (ii) minimum intervention period including follow-up of 12 months; (iii) body mass index: ≥ 25 kg/m²; (iv) comparing D + E vs. D or/and D + E vs. E or/and D vs. E; (v) assessment of "primary outcome" markers: body weight (BW), waist circumference (WC), waist-to-hip ratio (WHR), fat mass (FM) and "secondary outcome" markers: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triacylglycerols (TG), diastolic blood pressure (DBP), systolic blood pressure (SBP) and cardiorespiratory fitness (VO₂ max); (vi) participants with coronary heart disease were excluded; (vii) report post-intervention mean values (if not available change-from-baseline value scores were used) with standard deviation (or basic data to calculate these parameters: standard error or 95% confidence interval (CI)) according to the Cochrane Handbook [14]; and (viii) ≥ 19 years of age.

Risk of bias assessment

Full copies of studies were independently assessed for methodological quality by two authors (LS, GH) using the risk of bias assessment tool by the Cochrane Collaboration. The following sources of bias were detected: selection bias (random sequence generation, allocation concealment), performance/detection bias (blinding of participants and personnel, blinding of outcome assessment), attrition bias (incomplete data outcome) and reporting bias (selective reporting) (Figure 1) [14,15].

Data extraction and statistical analysis

The following data were extracted from each study: the first author's last name, publication year, study length (including follow-up), participant's sex and age, BMI, sample size, % T2D, intervention type, characteristics of dietary intervention, characteristics of exercise intervention, dropout rates, post-intervention mean values or change-from-baseline

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)
Anderssen et al. 1995	?	+	+	+	?	+
Andrews et al. 2011	+	+	+	?	+	+
Borg et al. 2002	+	?	+	+	?	?
Brekke et al. 2005	?	?	+	+	+	+
Christensen et al. 2013	+	+	+	+	+	+
Fogelholm et al. 2000	?	?	+	+	+	+
Foster-Schubert 2012	+	?	+	+	+	+
Messier et al. 2004	+	?	+	+	?	+
Messier et al. 2013	+	?	+	+	+	+
Pan et al. 1997	?	?	+	+	+	?
Pritchard et al. 1997	?	?	+	+	+	+
Racette et al. 2007	?	?	+	+	?	+
Skender et al. 1996	?	?	+	+	+	+
Snel et al. 2011	+	+	+	+	+	+
Stefanick et al. 1998	+	?	+	+	+	+
Villareal et al. 2011	+	?	+	+	+	+
Volpe et al. 2008	?	?	+	+	+	+
Wadden et al. 1998	?	?	+	+	+	?
Wing et al. 1988	?	?	+	+	+	+
Wing et al. 1998	?	?	+	+	+	+
Wood 1988	+	?	+	+	+	+
Wood et al. 1991	?	?	+	+	+	+

Figure 1 Risk of bias assessment tool. Across trials, information is either from trials at a low risk of bias (green), or from trials at unclear risk of bias (yellow), or from trials at high risk of bias (red).

value scores with corresponding standard deviation. Data extraction was performed by one author (LS).

Separate pairwise meta-analyses were first used to compare all lifestyle interventions. Network meta-analysis was then used to synthesize all the available evidence [16]. Network meta-analysis methods are extensions of the standard pairwise meta-analysis model which enable simultaneous comparison of multiple interventions whilst preserving the internal randomization of individual trials. They have the advantage of adequately accounting for the correlation in relative effect estimates from three-arm trials as well as providing a single coherent summary of all the evidence.

Pairwise meta-analyses

For each outcome measure of interest and for each pair of treatments, a random effects inverse variance meta-analysis was performed in order to determine the pooled effect of the intervention in terms of mean differences (MDs) between the post-intervention (or change-from-baseline) values of the different lifestyle interventions [14]. Data were pooled if outcomes were reported by at least three studies. Heterogeneity between trial results was tested with a standard χ^2 test. The I^2 parameter was used to quantify any heterogeneity: $I^2 = [(Q - d.f.)/Q] \times 100\%$, where Q is the χ^2 statistic and $d.f.$ is its degrees of freedom. A value for $I^2 > 50\%$ was considered to represent substantial heterogeneity [17]. As study characteristics were expected to differ, statistical heterogeneity was also expected and a random effects model was used to estimate MDs with 95% CIs. In addition, sensitivity analyses were planned to further elucidate the potential influence of heterogeneity due to different study characteristics on the outcome of the pairwise meta-analysis (such as study length, age of participants, risk of bias). Forest plots were generated to illustrate the study-specific effect sizes along with a 95% CI. To determine the presence of publication bias, we assessed the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors taking into account the recommendation by Sterne et al. [18], i.e. that testing for funnel plot asymmetry should only be conducted if the number of studies is ten or larger. Additionally, Begg's and Egger's regression tests were performed to detect small study effects [19,20].

Network meta-analyses

To account for the expected between-study heterogeneity, random effects network meta-analysis models were used. For each outcome, a common between-study heterogeneity parameter was assumed to reflect the variability between studies of all interventions.

Model fit was assessed by comparing the number of data points to the posterior mean of the residual deviance

[16] (these values should be similar in a well-fitting model). Pooled effect sizes from the network meta-analyses are presented as posterior medians and 95% credible intervals (CrI) (i.e. Bayesian equivalent of confidence intervals) in the appropriate units along with the estimated between-study heterogeneity and its 95% CrI. Treatments were ranked best, second best and third best based on their efficacy.

To assess sensitivity to the choice of random or fixed effects network meta-analysis models, the two models were compared using the deviance information criteria for each outcome [16,21] which account for both model fit and complexity. The fixed effects model was considered adequate when its deviance information criterion (DIC) was lower than the random effects model (differences >3 or 5 are considered meaningful) [16,21]. Mean differences for the fixed effects network meta-analysis (NMA) model are also presented for comparison.

Computation

For pairwise meta-analyses, data were analysed using the Review Manager 5.1 software, provided by the Cochrane Collaboration (<http://ims.cochrane.org/revman>). Network meta-analyses were conducted using Markov chain Monte Carlo (MCMC) simulation implemented with the open-source software WinBUGS, version 1.4.3 [22]. The WinBUGS code used is freely available online (program “TSD2-5aRE_Normal_id.odc” for the random effects models and “TSD2-5aFE_Normal_id.odc” for the fixed effects models) [16,23]. Minimally informative normal priors (with mean zero and variance 10,000) were used for all treatment effect parameters, and a uniform (0, 150) prior was used for the between-study standard deviation (heterogeneity) parameter. These priors were considered non-informative over the expected range of data. Sensitivity to the prior on the between-study heterogeneity was assessed by varying the upper bound of the uniform distribution, but there was no meaningful change in relative effects or overall conclusions.

Three MCMC chains were used to assess convergence using Brooks-Gelman-Rubin plots and by inspection of the trace plots [24]. Convergence was achieved after 20,000 iterations for all outcomes. Posterior summaries were then obtained from further simulation of 50,000 iterations in each of the three chains (150,000 in total), resulting in a small Monte Carlo error.

Treatments were ranked at each iteration (post-convergence) according to their efficacy, where the “best” treatment was the one with the most favourable outcome (which could be described by a higher or lower MD, depending on the outcome).

The potential for inconsistency was assessed by inspection of the network plots. Where there was a potential for inconsistency, i.e. where there were independent

sources of evidence informing direct and indirect estimates, Bayesian p values for the difference between direct and indirect evidence were calculated using the node split method [25,26] implemented in R through GeMTC [27], and direct and indirect estimates were compared.

Results

In order to ease interpretation of results, the following changes would be considered to be a benefit: BW, decrease; WC, decrease; FM, decrease; WHR, decrease; TC, decrease; LDL-C, decrease; HDL-C, increase; TG, decrease; DBP, decrease; SBP, decrease; VO_2 max, increase; altogether, 22 trials (24 reports) met the inclusion criteria and 21 of them were included in the quantitative analysis [28-52]. The detailed steps of the meta-analysis article selection process are given as a flow chart in Figure 2, and full search strategy for PUBMED and the Cochrane Trial Register is given in Additional file 1.

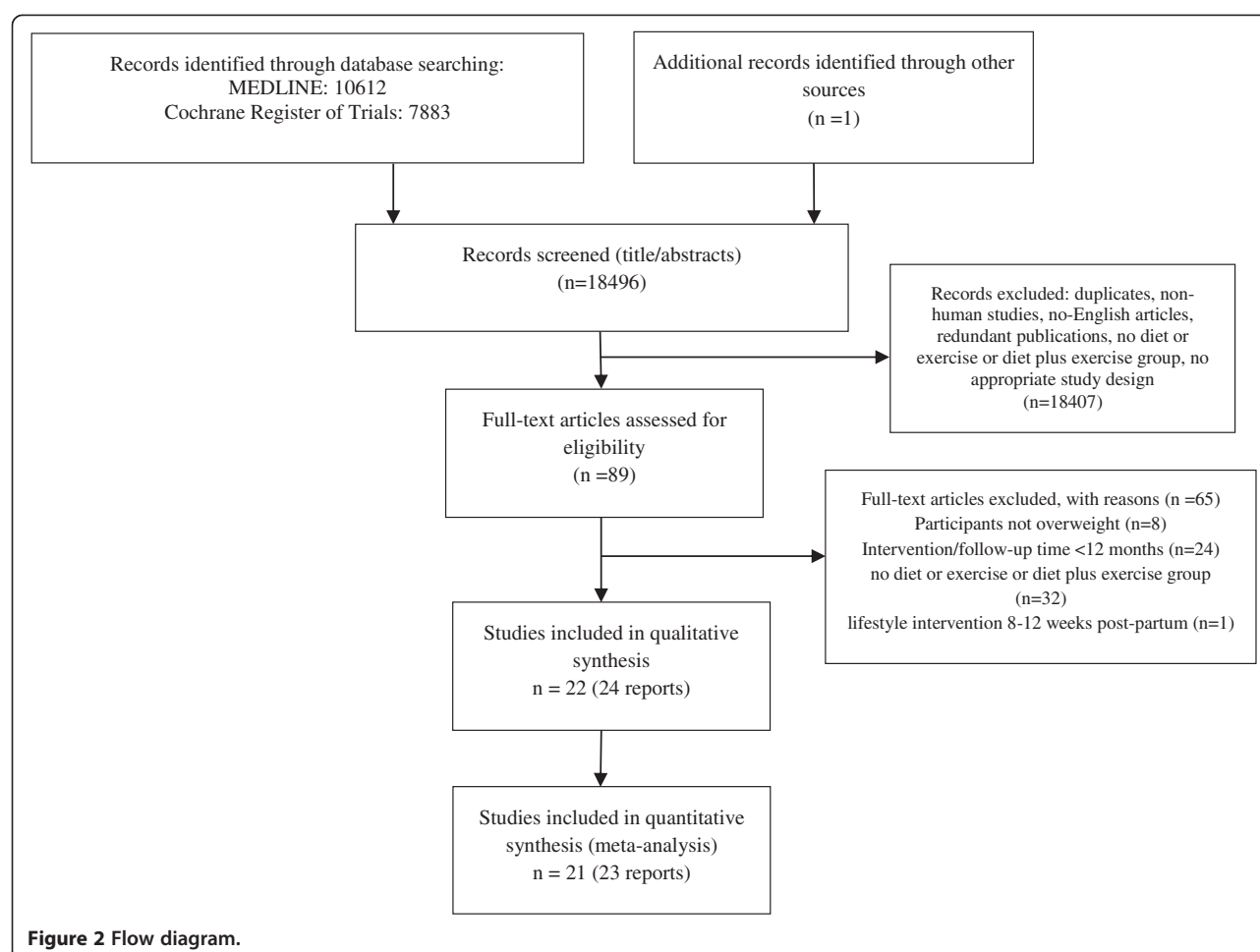
All studies included were randomized controlled trials (RCTs) with a duration ranging between 12 and 72 months, published between 1988 and 2013 and enrolling a total of 3,521 participants, 680 of them being participants with T2D. The mean age varied between 35 and 70 years and the BMI between 25.6 and 38.2 kg/m². Seventeen trials compared D + E vs. D, 11 compared D + E vs. E and 14 compared D vs. E. General study characteristics are summarized in Table 1. Regarding the dietary interventions, a major part of the included trials recommended energy-reduced low-fat diets ($\leq 30\%$ fat of total energy), low in saturated fat, and increased intakes of fruit, vegetables and fibre. Exercise prescription was partly supervised and included aerobic exercise (i.e. jogging, walking, flexibility, circuit training) and resistance training, overall 50%–85% of maximal heart rate.

The direct pairwise and network pooled estimate of effect size for the effects of D + E vs. D, D + E vs. E and D vs. E on anthropometric outcomes, blood lipids, blood pressure and cardiorespiratory fitness are summarized in Table 2.

Anthropometric outcomes/cardiorespiratory fitness

Diet + exercise vs. diet

The weighted mean difference in change of BW [MD: -1.38 kg (95% CI -1.98 to -0.79), $I^2 = 0\%$], WC [MD: -1.68 cm (95% CI -2.66 to -0.70), $I^2 = 0\%$], WHR [MD: -0.01 U (95% CI -0.02 to -0.01), $I^2 = 0\%$] and FM [MD: -1.65 kg (95% CI -2.81 to -0.49), $I^2 = 61\%$] was significantly more pronounced in the D + E group as compared to D, respectively. Furthermore, the D + E group revealed significantly more prominent increases in cardiorespiratory fitness (measured as VO_2 max) [MD: 3.61 ml/kg/min (95% CI 2.07 to 5.14), $I^2 = 88\%$] and HDL cholesterol [MD: 1.62 mg/dl (95% CI 0.28 to 2.95), $I^2 = 51\%$] as well as decreases in TG [MD: -10.08 mg/dl (95% CI -17.38



to -2.79), $I^2 = 0\%$] and DBP [MD: -1.20 mmHg (95% CI -2.26 to -0.15), $I^2 = 28\%$]. In contrast, changes observed for TC, LDL-C and SBP did not differ significantly between both groups.

Diet + exercise vs. exercise

Comparing D + E vs. E, a significantly more distinctive reduction in BW [MD: -4.13 kg (95% CI -5.62 to -2.64), $I^2 = 77\%$], WC [MD: -3.00 cm (95% CI -5.81 to -0.20), $I^2 = 69\%$], WHR [MD: -0.01 U (95% CI -0.02 to -0.00), $I^2 = 15\%$] and FM [MD: -3.60 kg (95% CI -6.15 to -1.05), $I^2 = 92\%$] could be observed in the D + E group. Rise of VO_2 max was significantly more pronounced in the D + E group as well [MD: 2.13 ml/kg/min (95% CI 1.52 to 2.74), $I^2 = 9\%$]. With respect to blood lipids, TC [MD: -11.36 mg/dl (95% CI -15.93 to -6.79), $I^2 = 0\%$], LDL-C [MD: -10.03 mg/dl (95% CI -14.28 to -5.78), $I^2 = 8\%$], DBP [MD: -2.06 mmHg (95% CI -3.39 to -0.72), $I^2 = 0\%$] and SBP [MD: -2.84 mmHg (95% CI -4.54 to -1.13), $I^2 = 0\%$] were reduced more substantially following combined D + E when compared to single E interventions. No significant differences could be observed for HDL-C and TG.

Diet vs. exercise

Following D vs. E comparisons, reductions in BW [MD: -2.93 kg (95% CI -4.18 to -1.68), $I^2 = 74\%$], FM [MD: -2.20 kg (95% CI -3.75 to -0.66), $I^2 = 82\%$], HDL-C [MD: -0.96 mg/dl (95% CI -1.88 to -0.04), $I^2 = 0\%$] and SBP [MD: -2.19 mmHg (95% CI -4.23 to -0.15), $I^2 = 25\%$] were significantly more pronounced in the D group. WC, WHR, TC, LDL-C and cardiorespiratory fitness were not affected in a different fashion by either D or E.

Network meta-analysis

Additional file 1: Figure S1 shows the network of included trials. The pooled estimates of effect size for the comparison of D + E vs. D vs. E using both direct and indirect evidence on anthropometric outcomes, blood lipids, blood pressure and cardiorespiratory fitness are summarized in Table 2.

Both D + E and D were significantly more effective in reducing BW and FM when compared to E alone. D + E turned out to be the most effective lifestyle intervention with respect to reduction of BW, WC, FM, TC, LDL-C, TG, DBP, SBP and increasing HDL-C. D + E resulted in

Table 1 General study characteristics

Reference	Sample size Baseline BMI (kg/m ²) % diabetics	Age (years) Female (%)	Duration of the active intervention (follow-up)	Study design	Dietary intervention Exercise prescription	Dropout
Anderssen et al. [40] Reseland et al. [41]	166 28.9 0%	40 0%	12 months (0 months)	D + E vs. D vs. E	D: increased fish, fruit and vegetables and fibre, reduce intake of sugar and SFA, no heavy evening meal E: supervised weekly, aerobic training (strength, flexibility, circuit training, jogging), 60%–80% of peak heart rate	D + E: 3% D: 5% E: 9%
Andrews et al. [42]	494 31.55 100%	60 35%	12 months (0 months)	D + E vs. D	D: aimed at enabling patients to lose 5%–10% of their initial body weight, based on UK dietary guidelines E: asked to do at least 30-min brisk walking on at least 5 days per week	D + E: 2% D: 1%
Borg et al. [43]	82 32.9 n.d.	42.6 0%	8 months (23 months)	D + E vs. D	D: low-fat diet E: supervised weekly in group, reached 50%–60% of MHR, included two groups: (1) walking, expended 1,000 kcal per week, and (2) walking, expended 2,000 kcal per week	D + E: 18% D: 1%
Brekke et al. [44]	49 25.6 100%	43 33%	12 months (12 months)	D + E vs. D	D: Nordic Nutrition Recommendation in addition increase of low GI food E: the goal to increase physical activity through walking or other more intensive activities for at least 30 min, four to five times per week	D + E: 17% D: 4%
Christensen et al. [52]	28 37.05 0%	63 81.3	17 months (0 months)	D vs. E	D: the goal of the dietary intervention was to produce and maintain a weight loss of at least 10% E: exercise intervention consisted of a warm-up phase (10 min), a circuit training phase (45 min) and a cool down/stretching phase four periods of 12 weeks and one period of 4 weeks (total 52 weeks). The aim was to gradually translate the intervention from facility-based exercises to home-based exercises	D: 14% E: 19%
Fogelholm et al. [45]	82 34 0%	35 100%	13 months (11 months)	D + E vs. D	D: low-fat diet E: supervised weekly in group, reached 50%–60% of MHR, included two groups: (1) walking, expended 1,000 kcal per week, and (2) walking, expend 2,000 kcal per week	D + E: 2% D: 3%
Foster-Schubert et al. [46]	351 28.6 0%	58 100%	12 months (0 months)	D + E vs. D vs. E	D: total daily energy intake 1,200–2,000 kcal/day on baseline weight <30% fat and 10% reduction in body weight by 6 months with maintenance thereafter for 12 months E: ≥45 min of moderate-to-vigorous intensity exercise, 5 days/week (225 min/week) for 12 months; supervised; 70%–85% of MHR	D + E: 8% D: 11% E: 9%
Messier et al. [51] Nicklas et al. [47]	238 34.3 9%	68.7 73%	18 months (0 months)	D + E vs. D vs. E	D: the goal of intervention was to produce and maintain an average weight loss of 5% baseline body weight. The intervention was divided into three phases: intensive, transition and maintenance E: exercise consisted of an aerobic phase, a resistance training phase, a second aerobic phase and a cooling down phase	D + E: 24% D: 23% E: 20%

Table 1 General study characteristics (Continued)

Messier et al. [50]	454	66	18 months	D + E vs. D vs. E	D: diet was based on partial meal replacements, including up to two meal replacement shakes per day; for the third meal, participants followed a weekly menu plan and recipes that were 500 to 750 kcal, low in fat and high in vegetables; initial diet plan provided an energy intake deficit of 800 to 1,000 kcal/day E: exercise was conducted for 1 h on 3 days/week for 18 months; programme consisted of aerobic walking (15 min), strength training (20 min), a second aerobic phase (15 min) and cool down (10 min)	D + E: 11%
	33.6	72%	(0 months)			D: 15%
	13%					E: 11%
Pan et al. [48]	397	44.4	72 months	D + E vs. D vs. E	D: caloric intake at 25–30 kcal/kg of BW, increased vegetable intake and reduced intake of sugars, using individual goals E: increased the amount of exercise at least 1 U/day and U/day for those less than 50 years old with no evidence of heart disease or arthritis. The rate of increase and type of exercise depending on age, past exercise pattern and existence for heart problem other than IGT	D + E: 8%
	25.6	47%	(0 months)			D: 8%
	0%, 100% IGT					E: 8%
Pritchard et al. [39]	39	44.25	12 months	D vs. E	D: low-fat diet E: aerobic exercise; minimum participation was three sessions of 30 min per week. 65%–75% of MHR was recommended to achieve maximum weight loss	D: 0%
	29.1	0%	(0 months)			E: 0%
	0%					
Racette et al. [38]	45	57.2	12 months	D vs. E	D: decrease energy intake by 16% for the initial 3 months and by 20% for the remaining 9 months; macronutrient composition was flexible E: the goal of the E intervention was to induce an energy deficit comparable to the CR intervention by increasing daily energy expenditure through exercise without changing caloric intake. Exercise physiologists and trainers worked with ex-participants individually to establish and monitor their exercise routines	D: 4%
	37.2	63%	(0 months)			E: 4%
	0%					
Skender et al. [37]	61	45	12 months	D + E vs. D vs. E	D ^a : help your heart eating plan; well-balanced, low-cholesterol eating plan E: supervised weekly although group brisk walking at a level of felt "vigorous" not "strenuous", 45 min, 4–5 times/week	D + E: 50%
	35	48%	(12 months)			D: 65%
	0%					E: 42%
Snel et al. [36]	27	57.5	4 months	D + E vs. D	D: 4 months: 450 kcal/day (consisting of three sachets of Modifast); weight maintenance: 1,800 kcal E: 4 days training at home for 30 min at 70% of maximum aerobic capacity on a cyclo-ergometer and 1 h in hospital training under the supervision of a physiotherapist	D + E: 0%
	37	48%	(14 months)			D: 0%
	100%					
Stefanick et al. [35]	276	56.9/47.8	12 months	D + E vs. D vs. E	D: NCEP step 2 diet E: supervised weekly, aerobic exercise =16-km jogging per week	D + E: 3%
	26.3/27	48%	(0 months)			D: 3%
	0%					E: 3%

Table 1 General study characteristics (Continued)

Villareal et al. [34]	80	70	12 months	D + E vs. D vs. E	D: balance diet with an energy deficit of 500 to 750 kcal. The diet contained 1 g of high-quality protein/kg of BW per day E: three supervised exercise training sessions per week. Each session was 90 min in duration and consisted of aerobic and resistance exercise and exercise to improve flexibility and balance	D + E: 11%
	37	61%	(0 months)			D: 15%
	n.d.					E: 12%
Volpe et al. [33]	90	44.4	6 months	D + E vs. D vs. E	D: intensive (weekly) nutritional classes (1–3 months) E: supervised training on Nordic Track™ indoor skiing apparatus, 3–4 days per week, 30 min for 6 weeks	D + E: n.d.
	30.5/35.3	51%	(6 months)			D: n.d.
	0%					E: n.d.
Wadden et al. [32]	77	42	12 months	D + E vs. E	D: conventional diet with 1,200–1,500 kcal/day E: supervised weekly in a group, 1 h, two times per week, included in three exercise groups: (1) aerobic, (2) strength and (3) combined training	D + E: 22%
	36.5	100%	(0 months)			E: 22%
	0%					
Wing et al. [29]	114	45.5	24 months	D + E vs. D vs. E	D: participants were asked to follow an 800–100 kcal/day diet, with 20% of calories as fat, exactly as prescribed for 1–8 weeks of the programme. Gradually more flexible with calorie goals adjusted to 1,200–1,500 kcal/day at week 16. Subject attended weekly group meetings for the first 6 months E: supervised by exercise physiologists weekly in a group. Mainly brisk walking, 3 miles, five times per week; total activity gradually increased to 1,500 kcal per week	D + E: 20%
	35.9	79%	(0 months)			D: 5%
	0%					E: 16%
Wing et al. [30]	30	55.56	12 months	D + E vs. D	D: daily calorie goal designed to produce approximately 1 kg/week weight loss. Low-fat diet E: all participants exercised twice a week as a group and once a week on their own, with each exercise session lasting approximately 1 h	D + E: 13%
	38.2	70%	(0 months)			D: 0%
	100%					
Wood et al. [49]	152	39.1/40.3	12 months	D + E vs. D	D ^b : NCEP step 1 diet E: aerobic exercise (brisk walking and jogging) that met 3 days a week, 60%–80% of MHR for 25–45 min per time (by the fourth month of the study)	D + E: 14%
	27.9/30.7	48%	(0 months)			D: 13%
	0%					
Wood et al. [28]	89	44.1	12 months	D vs. E	D: individual prescription designed to reduce baseline total body fat by one third over a 9-month period E: supervised exercise programme and individual prescriptions based on estimates of the amount of energy necessary to decrease total body fat progressively by one third over 9 months	D: 4%
	n.d.	0%	(0 months)			E: 2%
	0%					

BMI body mass index, *BW* body weight, *CR* caloric restriction, *D* diet, *E* exercise, *GI* glycaemic index, *IGT* impaired glucose tolerance, *MHR* maximal heart rate, *NCEP* National Cholesterol Education Program, *SFA* saturated fat, *UK* United Kingdom; *n.d.* no data.

^aSkender et al. [37]: 50% CH, 30% F, 20% P, low cholesterol.

^bWood et al. [49]: 55% CH, 30% F, <10% SFA, <300 mg/day.

Table 2 Estimates (direct pairwise and network meta-analysis, random effects models) of effect size (95% confidence intervals/95% credible intervals) expressed as mean difference for the effects of diet + exercise vs. diet, diet + exercise vs. exercise and diet vs. exercise on anthropometric outcomes, blood lipids, blood pressure and cardiorespiratory fitness and between-study heterogeneity variance (τ^2/τ)

Outcomes	No. of studies	Sample size	MD	95% CI	τ^2	I^2	MD	95% CrI	τ	95% CrI
D + E vs. D										
BW (kg)	17	2,317	-1.38	[-1.98, -0.79]	0.00	0%	-1.38	[-2.62, -0.17]	2.06	[1.37, 2.96]
WC (cm)	8	1,124	-1.68	[-2.66, -0.70]	0.00	0%	-1.69	[-3.32, -0.20]	1.36	[0.07, 3.43]
FM (kg)	9	1,012	-1.65	[-2.81, -0.49]	1.95	61%	-1.89	[-3.44, -0.43]	2.08	[1.29, 3.24]
WHR (U)	6	646	-0.01	[-0.02, -0.01]	0.00	0%	-0.01	[-0.05, 0.03]	0.06	[0.04, 0.10]
TC (mg/dl)	9	1,175	-2.19	[-7.84, 3.46]	54.09	62%	-2.51	[-7.61, 2.29]	5.42	[0.86, 10.84]
LDL-C (mg/dl)	8	1,147	-0.93	[-6.14, 4.27]	45.03	65%	-1.54	[-6.16, 3.14]	5.29	[1.26, 9.97]
HDL-C (mg/dl)	9	1,175	1.62	[0.28, 2.95]	2.46	51%	-1.29	[-1.38, 3.86]	4.03	[2.38, 6.33]
TG (mg/dl)	9	1,175	-10.08	[-17.38, -2.79]	0.00	0%	-9.90	[-19.98, -0.96]	7.28	[0.37, 19.4]
DBP (mmHg)	7	1,099	-1.20	[-2.26, -0.15]	0.74	28%	-1.10	[-2.34, 0.01]	0.92	[0.04, 2.45]
SBP (mmHg)	7	1,099	-0.24	[-1.45, 0.97]	0.00	0%	-0.39	[-1.89, 1.01]	0.87	[0.04, 2.62]
VO ₂ max (ml/kg/min)	6	810	3.61	[2.07, 5.14]	4.19	88%	3.75	[2.28, 5.32]	1.94	[1.13, 3.18]
D + E vs. E										
BW (kg)	9	1,350	-4.13	[-5.62, -2.64]	4.36	77%	-4.32	[-5.74, -2.90]	2.06	[1.37, 2.96]
WC (cm)	3	409	-3.00	[-5.81, -0.20]	5.24	69%	-3.45	[-5.32, -1.23]	1.36	[0.07, 3.43]
FM (kg)	5	690	-3.60	[-6.15, -1.05]	8.82	92%	-3.87	[-5.61, -2.18]	2.08	[1.29, 3.24]
WHR (U)	4	420	-0.01	[-0.02, -0.00]	0.00	15%	-0.007	[-0.06, 0.04]	0.06	[0.04, 0.10]
TC (mg/dl)	4	420	-11.36	[-15.93, -6.79]	0.00	0%	-7.50	[-13.47, -1.39]	5.42	[0.86, 10.84]
LDL-C (mg/dl)	4	420	-10.03	[-14.28, -5.78]	2.22	8%	-5.90	[-11.39, -0.23]	5.29	[1.26, 9.97]
HDL-C (mg/dl)	4	420	-0.34	[-2.82, 2.14]	6.80	76%	0.17	[-3.14, 3.32]	4.03	[2.38, 6.33]
TG (mg/dl)	4	420	-11.18	[-26.99, 4.62]	138.3	38%	-13.34	[-25.92, -2.12]	7.28	[0.37, 19.4]
DBP (mmHg)	4	420	-2.06	[-3.39, -0.72]	0.00	0%	-2.22	[-3.93, -0.74]	0.92	[0.04, 2.45]
SBP (mmHg)	4	420	-2.84	[-4.54, -1.13]	0.00	0%	-2.70	[-4.57, -0.85]	0.87	[0.04, 2.62]
VO ₂ max (ml/kg/min)	5	645	2.13	[1.52, 2.74]	0.05	9%	2.24	[0.57, 3.90]	1.94	[1.13, 3.18]
D vs. E										
BW (kg)	13	1,638	-2.93	[-4.18, -1.68]	3.70	73%	-2.93	[-4.20, -1.66]	2.06	[1.37, 2.96]
WC (cm)	4	539	-1.75	[-4.12, 0.62]	4.80	71%	-1.76	[-3.48, 0.44]	1.36	[0.07, 3.43]
FM (kg)	9	964	-2.20	[-3.75, -0.66]	4.66	82%	-1.97	[-3.45, -0.45]	2.08	[1.29, 3.24]
WHR (U)	4	414	-0.00	[-0.01, 0.01]	0.00	16%	0.002	[-0.05, 0.05]	0.06	[0.04, 0.10]
TC (mg/dl)	7	665	-3.91	[-8.11, 0.30]	9.04	22%	-4.98	[-10.22, 0.64]	5.42	[0.86, 10.84]
LDL-C (mg/dl)	7	665	-3.19	[-6.85, 0.48]	6.45	21%	-4.36	[-9.25, 0.70]	5.29	[1.26, 9.97]
HDL-C (mg/dl)	7	665	-0.96	[-1.88, -0.04]	0.00	0%	-1.12	[-4.06, -1.76]	4.03	[2.38, 6.33]
TG (mg/dl)	7	665	-3.80	[-12.21, 4.62]	0.00	0%	-3.44	[-13.99, 6.65]	7.28	[0.37, 19.4]
DBP (mmHg)	6	573	-1.33	[-3.00, 0.35]	11.19	37%	-1.12	[-2.67, 0.31]	0.92	[0.04, 2.45]
SBP (mmHg)	6	578	-2.19	[-4.23, -0.15]	2.07	25%	-2.31	[-4.10, 0.51]	0.87	[0.04, 2.62]
VO ₂ max (ml/kg/min)	6	677	-1.16	[-2.42, 0.09]	2.17	80%	-1.15	[-3.16, 0.04]	1.94	[1.13, 3.18]

BW body weight, CI confidence intervals, D diet, DBP diastolic blood pressure, D + E diet and exercise, E exercise, FM fat mass, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, SBP systolic blood pressure, TC total cholesterol, TG triacylglycerols, VO₂ max maximal oxygen uptake, WC waist circumference, WHR waist-to-hip ratio.

a high (>75%) probability to be the best for most outcomes. There is greater uncertainty regarding which treatment is the best for HDL-C, although again D + E yielded the highest probability of being the best. D turned

out be the second effective lifestyle intervention for BW, WC, FM, TC and DBP (>75% probability).

There was potential for inconsistency in the networks for all outcomes except WHR (Additional file 1: Figure S1).

There was some evidence of inconsistency for the outcome LDC (p value = 0.02) although this might be due to chance since several p values for inconsistency are being calculated (Additional file 1: Table S8). After inspection of the evidence on this outcome we did not identify a reason for this apparent inconsistency.

Risk of bias

The dropout rates ranged from 0% to 65%, with 11 out of 22 trials reporting dropout rates <10% (Table 1). Pairwise meta-analysis resulted in no significant drop-out differences (p = 0.37) between D + E (122/1,257) vs. D (111/1,152), D + E (79/714) vs. D (82/715) (p = 0.96) and D + E (87/701) vs. D (84/706) (p = 0.34). Eight trials (nine reports) reported random sequence generation [34,35,42,43,46,47,50-52], and only two trials reported allocation concealment [42,52]. None of the studies reported blinding of volunteers towards mode of intervention (not possible in diet/exercise trials), and four trials (five reports) appear to have adequate blinding of outcome assessment [46,47,50-52]. High risk of bias was defined as fewer than three out of a maximum yield of six low risk of bias items using the risk of bias assessment tool from the Cochrane Collaboration. Only seven low risk of bias trials (eight reports) were identified [34,35,42,46,50-52], and sensitivity analyses were performed for studies with a low risk of bias (Additional file 1: Table S1).

Sensitivity analysis

Sensitivity analyses (pairwise meta-analysis) were performed for obese, study length ≥ 24 months, older participants (age: ≥ 50 years) and low risk of bias studies. Comparison of long-term vs. short-term trials resulted in smaller reductions in body weight (Additional file 1: Tables S2-S3). The results of the primary analysis (D + E vs. D and D vs. E) could be confirmed including only obese participants (Additional file 1: Table S4), except for WC and VO₂ max. Including only participants ≥ 50 years of age (Additional file 1: Table S5), results of the primary analysis could not be confirmed regarding anthropometric outcomes when comparing D + E vs. D (only four to eight trials available), whilst the results of the main analysis for D vs. E were not affected. Sensitivity analyses excluding trials with a high risk of bias changed some summary estimates (D + E vs. D) for anthropometric outcomes and became statistically non-significant (with the exception of D vs. E comparisons).

Fixed vs. random effects models

Results of the pairwise meta-analyses with a fixed effects model are presented in Additional file 1: Table S6. Overall, the results of the main analysis could be confirmed in the fixed pairwise sensitivity analyses. However comparing D vs. E resulted in a significant reduction in WC,

TC and LDL-C in the fixed effects model, which was not observed in the random effects model, that were more pronounced in the D group. However, we note that the between-study heterogeneity was moderate to high for these outcomes and therefore the fixed effects model may not be appropriate.

In the NMA, comparing the DIC for fixed and random effects models suggests that the fixed effects model might be appropriate for DBP, SBP, TC, TG and WC (Additional file 1: Table S7). The estimated treatment effects using fixed effects network meta-analyses are given in Additional file 1: Table S6. Some differences could be observed between the random and fixed effects models. In the fixed effects model, comparing D vs. E resulted in a significant reduction in WC, TC, LDL-C, SBP and VO₂ max, favouring D (with the exception of VO₂ max), compared to the random effects model. Furthermore, the fixed effects model showed a significant reduction in WHR, comparing D + E vs. D/E, and increase in HDL-C compared to E. Again, we note that the fixed effects model was not always considered suitable (Additional file 1: Table S7).

Publication bias

Begg's and Egger's regression tests provided no evidence of substantial publication bias (data not shown). Funnel plots were generated only if specific outcome measures were provided by at least ten different trials (some trials provide more than one data point for analysis as they report results for subgroups: men and women) [18]. The plot with respect to effect size change for body weight in response to D + E vs. D vs. E indicates little asymmetry (Additional file 1: Figures S2-S3). Thus, publication bias cannot be completely excluded as a factor affecting the results of the present meta-analysis.

Discussion

The primary findings of this systematic review and meta-analysis of long-term trials are that a combination of D + E is more effective in reducing BW compared to either D or E in overweight and obese participants. Furthermore, improvements in WC, WHR and FM were significantly more pronounced following D + E compared to either D or E. The combination of D + E is also the most powerful lifestyle intervention to reduce blood lipids and blood pressure. Compared to E, a dietary intervention resulted in significantly greater reductions in body weight, fat mass and systolic blood pressure. Pooling both direct and indirect evidence on D + E, D and E via network meta-analysis demonstrated that D + E was the most efficacious exercise intervention regarding its impact on BW, WC, FM, TC, LDL-C, TG, DBP and VO₂ max.

The findings of this meta-analysis, i.e. diet being significantly more effective in reducing BW than exercise, are supported by results of a 2006 Cochrane review [11]

and are consistent with results of another meta-analysis [53]. Therefore, caloric restriction appears the most powerful method for achieving weight loss in overweight and obese people. The amount of weight loss yielded by diet and exercise can be compared to the corresponding results of the most effective pharmacological interventions such as orlistat (4.12/3.1 kg at 12 months) [54,55]. A recent systematic review concluded that orlistat, lorcaserin and phentermine/topiramate ER when used as an adjunct to lifestyle intervention, could induce a clinically relevant ($\geq 5\%$) 12-month weight loss [56]. The interpretation of our network meta-analysis is restricted by the fact that none of the trials evaluated the impact of their interventions on clinical outcomes. It should be noted, however, that no anti-obesity drugs have been shown to have a favourable effect on CVD morbidity and mortality as well [57]. Furthermore anti-obesity drugs were associated with increased risk of total adverse events, tachycardia, gastrointestinal disease, hypertension and mouth dryness [58].

Previous studies reported that a 5% reduction of BW is associated with a reduced risk of T2D incidence and other metabolic disorders. A 5-kg weight loss over time could account for a 55% reduction in the risk of diabetes over the mean of 3.2 years of follow-up in a high-risk population [59]. Regarding visceral adipose tissue, a comprehensive meta-analysis of cohort studies showed that a 1-cm increase in WC and a 0.01-U increase in WHR are associated with a 2%–5% increase in risk of future CVD [60]. Applying this data to the results of the present meta-analysis, D + E would be associated with a CVD risk reduction of ~3%–6%. Improvements on anthropometric outcomes were more distinct in younger participants compared with older which might be explained by the fact that younger participants were able to perform more intense exercise sessions. However, since not all trials applied supervised exercise, no definitive explanation can be given.

With respect to blood lipids, a meta-analysis of 70 studies indicate that each kilogram of weight loss was associated with a 1.9 mg/dl decrease in TC and a 0.77 mg/dl decrease in LDL-C, respectively [61]. Furthermore, RCTs showed that especially aerobic exercise was associated with an increase in HDL-C [62]. These associations could be confirmed in the present meta-analysis.

The predominant dietary intervention implemented in the included trials was either an energy-restricted low-fat diet or an energy-balanced moderate-fat diet. In general, the composition of diets was approximately at least 500 kcal below the estimated energy need, and fat intake was $\leq 30\%$ of total energy content. In the D + E and D trials, 1,200 kcal for women and 1,500 kcal for men were generally prescribed. In the dietary intervention trials, general guidelines for physical activity were recommended.

However, in the D + E trials, specific goals for physical activity/exercise were implemented. Network meta-analyses provides evidence that a combination of aerobic and resistance training should be recommended in the prevention and treatment of overweight, obesity and associated diseases [4,10].

Cardiorespiratory fitness is associated with cardiovascular mortality and cancer in men and women [63,64]. A pooled analysis investigating the effects of cardiorespiratory fitness on all-cause mortality and cardiovascular events demonstrated that a 1-unit increase in metabolic equivalents was associated with a 13% and 15% reduction in risk of all-cause mortality and coronary heart disease (CHD)/CVD, respectively [65]. Transferring this data to the results of the present meta-analysis, D + E reduced the risk of all-cause mortality by 14% and of CVD/CHD by 16% and approximately 8% (mortality) and 9% (CVD/CHD) following applications of single lifestyle interventions, respectively. A possible explanation of the superior effect of D + E on cardiorespiratory fitness could be the greater weight loss induced by caloric restriction.

The present systematic review has several limitations. A major limitation is that no search for unpublished studies and any additional data sources and strategies (author contacts, trial registers) was performed. Another limitation is the fact that not all potential effect modifiers were accounted for. Often participants in different arms of trials did not receive equal numbers of contacts. It could be argued that contact time rather than the specific elements of the intervention affected participant's weight and cardiovascular risk factor outcomes. Heterogeneity could be observed for some outcome parameters, probably introduced by differences between trials, including different D + E regimens. Publication bias cannot be excluded to affect the results on any meta-analysis; however, formal statistical testing did not suggest publication bias for the current analysis. Had there been evidence of publication bias and if more studies were available, regression techniques could be used to adjust for this [66].

Although many studies included in our analysis had a substantial dropout rate (see Table 1), intention-to-treat analyses were generally not conducted. However, dropouts were generally similar for all intervention groups. Taken together, 2/3 of the included trials were judged as being at high risk of bias. Therefore, the results should be interpreted in a conservative manner. Strengths of this research include the application of the network meta-analysis, as well as the fact that there was no evidence of inconsistency for most outcomes, and an overall sample size of 3,521 participants.

Conclusions

The present network meta-analysis provides moderate-quality evidence that D + E induces moderate long-term

weight loss and reduces blood lipids and blood pressure when compared to E or D as single interventional measures, respectively. In addition, the evidence suggests moderate superiority of D over E regarding anthropometric outcome parameters. The current findings seem to be clinically relevant for public health, in particular for encouraging a combination of diet and exercise for primary prevention of overweight and obesity. Future trials should investigate the long-term effects of different training modalities (aerobic, resistance or combined training) in combination with dietary interventions for the prevention and treatment of overweight and obesity.

Additional file

Additional file 1: Full search strategy: PUBMED. Table S1: sensitivity analysis (low risk of bias). **Table S2:** sensitivity analysis (study length: ≥ 24 months). **Table S3:** sensitivity analysis (study length, < 24 months). **Table S4:** sensitivity analysis (obese participants). **Table S5:** sensitivity analysis (participants ≥ 50 years of age). **Table S6:** sensitivity analyses (direct pairwise and network meta-analysis, fixed effects models). **Table S7:** model fit statistics for the random and fixed effects network meta-analysis models. **Table S8:** estimates of the effects of diet + exercise vs. exercise from direct and indirect evidence in node split models. **Figure S1:** network graph. **Figure S2:** funnel plot for body weight (diet + exercise vs. diet). **Figure S3:** funnel plot for body weight (diet + exercise vs. exercise). **Figure S4:** funnel plot for body weight (diet vs. exercise).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LS extracted the data. LS, SD and GH conducted the data analysis, interpreted the results and drafted and finalized the manuscript. All authors read and approved the final manuscript.

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