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MASTER THESIS

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The effect of reducing meat consumption on selected biomarkers of type 2 diabetes and cardiovascular disease: A systematic review and meta-analysis

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This thesis is dedicated to my parents. Hopefully this review will make my arguments a bit more valid, and that consuming more plant-based food isn't as bad as it seems.

Bergen, September 2022

Marthe Synnøve Fiskaa Mila

ABSTRACT

Nearly 500 million people are living with T2DM and 562 million are affected by CVD worldwide (1). Countries with high socio-economic development tend to have more cases of T2DM and CVD, which is seen to correlate with the amount of meat-intake per capita (2).

The aims of this study were to conduct a systematic review and meta-analysis of randomized controlled trials to assess and thoroughly research the effect of a meat-reduced diet in adults on biomarkers of T2DM and CVD. Biomarkers chosen for this review were HbA1c, LDL-cholesterol, and HDL-cholesterol.

A literature search was conducted in PubMed, Embase, and Cochrane Library which retrieved 3.658 articles, in which 10 articles met the eligibility criteria. Meta-analysis on the extracted data from the included articles was performed using STATA, which provided results on HbA1c outcomes, LDL-cholesterol outcomes, and HDL-cholesterol outcomes. Sub-group analyses of meta-analysis (HbA1c, LDL-cholesterol, and HDL-cholesterol) on study duration, diabetes status of the participants, and age-group were provided.

The main findings from this systematic review and meta-analysis indicate notable evidence of reduced LDL-cholesterol and HbA1c levels when reducing the consumption of red, and processed meat – but there was no evidence of a statistical significant effect on HDL-cholesterol levels. These findings support the nutritional recommendations from World Health Organization (WHO) and Norwegian Health Informatics (NHI) to reduce meat consumption in relation to the increasing risk of developing type 2 diabetes and cardiovascular disease (3-5).

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ABBREVIATIONS

T2DM	Type 2 diabetes mellitus
CVD	Cardiovascular disease
NCDs	Non-communicable diseases
MUFA	Monounsaturated fatty-acids
SFA	Saturated fatty-acids
LDL-c	Low-density lipoprotein cholesterol
HDL-c	High-density lipoprotein cholesterol
TC	Total cholesterol
S-cholesterol	Serum cholesterol
IARC	International Agency for Research on Cancer
NHI	Norsk Helseinformatikk/Norwegian Health Informatics
HbA1c	Hemoglobin-A1c
FPG	Fasting glucose plasma
OGTT	Oral glucose tolerance test
SD	Standard deviation
SE	Standard error
WHO	World Health Organization
PICO(T)	Population, Intervention, Comparison, Outcome, (Time frame)
RCT	Randomized controlled trial
MSFM	Marthe Synnøve Fiskaa Mila (review author)
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
INT	Intervention
CONT	Control
Diff	Difference
QA	Quality assessment
MD	Mean difference
CI	Confidence interval
IQR	Inter-quartile range
RoB	Risk of bias

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The effect of reducing meat consumption on selected biomarkers of type 2 diabetes and cardiovascular disease: A systematic review and meta-analysis

1.0 Introduction

This thesis is a systematic review and meta-analysis on meat reduction on specific biomarkers of cardiovascular disease and type 2 diabetes mellitus. The introduction consists of a general background on dietary, environmental, and health-related factors in relation to meat consumption. Following, an introduction of the relevance of meat consumption to selected non-communicable diseases and how that is commonly assessed using biomarkers will be presented. Lastly, a justification for the study objectives will be given.

1.1 Background

Consumers are increasingly encouraged to reduce meat consumption, as overconsumption of meat is the major driver of greenhouse gas emission (6), and several studies have shown a link between the consumption of red and processed meat, and the development of cardiovascular diseases (CVD) and type 2 diabetes mellitus (T2DM) (7-9). T2DM is one of the top leading causes of mortality worldwide after CVD, and they are both categorized as so-called non-communicable diseases (NCDs), meaning that both environmental, life-style and genetic factors interplay (10, 11). Interventions within nutrition are crucial to reduce the risk of NCDs (11). Despite the adverse impact of meat consumption on health and the environment, meat consumption has steadily increased both in Europe and globally in the last 50 years, increasing from 23.1 kg in the 1960s to 42.2 kg in 2011 per person. It is estimated that the total meat consumption is twice the average of the recommended amount per capita (12). Meat and meat-products have been the main source of protein for humans since prehistoric times (13, 14). The consumption of animal protein is rising exponentially with higher income and population growth, and it is expected to increase by nearly 2% by the end of 2022 (15), and 12% by 2029 globally. Currently, the amount of pork consumed annually in the EU is estimated to be at 36 kg per capita, and recent statistics from 2022 estimated an intake of 40 kg of beef per capita annually in Argentina (15).

There is also expected to be changes in animal protein sources, where it is estimated that up to 41% of protein intake will come from poultry, while intake of other meat sources such as beef, pork, and sheep will continue to decrease by 2030 (16). As of Norway, in a report from 2018 the Norwegian Directorate of Health pointed out that the average Norwegian consumed up to 75 kg of meat annually– twice as much as the rest of the EU (3, 17).

1.1.1 The role of meat in diet

Meat has a central role when it comes to food culture and tradition in many countries across the globe, and it has been a part of our diet for thousands of years (15). In terms human development, consuming meat has contributed to longevity by evolving the brain, esophagus, and gastrointestinal tract (18). However, meat consumption patterns are different around the world and is seen to be highest in high-income countries, with Europe, North America, and Australia taking the lead. We can see that the consumption of meat is increasing parallelly with the worlds growing economy (2). On the other hand, World Health Organization (WHO) has stated that over 820 million people were food insecure and lacked access to healthy, nutritious, and sufficient food including animal-sourced foods in 2021 (19). Due to the lack of food security for hundreds of millions globally, agriculture involving local farming of livestock and cattle is vital for people to make a living and ensure nutritious food (20).

1.1.2 Meat consumption and the environment

Current estimates show that 50% of 71% habitable land is used for agriculture, with its largest share (roughly 40 million km^2) being used for livestock production, and meat production in general is forecasted to be doubled by 2050 (21). The land used for agriculture includes food production, grazing areas, and habitat for animals. Looking at these numbers and the large habitable land areas taken up by livestock, it would be logical to assume that most of the world's protein and nutrient supply would come from these productions. However, only 37% of the world’s protein supply comes from livestock, whereas the remaining comes from plant-based foods (2). Agriculture is affecting the environment, both climate and natural resources, and it is human-made due to rapid population growth (22). Biodiversity and land is threatened as a result of human activity, and in the last five centuries it is estimated that over 900 species have gone extinct due to these events (23, 24). Agriculture and livestock have a massive impact on destruction of land, and thus degradation of both biodiversity and soil. Also, almost 27% of the

earth's greenhouse gas emissions are from food production alone, and livestock contributes to a third of this (25). Crop fields, transportation, water-use, processing, and storage of foods account for the remaining greenhouse gas emissions from food production (2).

1.1.3 Meat and health

Meat is an important source for nutrients, including protein and key micro-nutrients such as iron and B-complex vitamins (26, 27). B-complex vitamins includes niacin, B12, riboflavin, B6, and thiamin. Vitamin B12 is one important component of this complex, and it is mainly found in food sources like meat and some algae (Nori) (28). However, it is known that different ways of handling meat can also break down some of the B-complex vitamins. This is because vitamin B12 is a water-soluble vitamin and heat-sensitive, and therefore cooking it for too long on high temperature can result in less nutritious meat (27). A study published by Harvard Chan School of Public Health's Department of Nutrition has found a correlation between high-temperature cooking of both poultry and red meat, and risks of developing T2DM (29). An important source in a meat-reduced diet is dietary fiber, which are non-digestible carbohydrates and lignin of plant origin (30, 31). This is a group of two components; insoluble and soluble fiber, and it is recommended to consume 25-38 g/day (32). Legumes, vegetables, nuts, seeds, and fruits are examples of healthy and beneficial dietary fibers, and consuming a diet high in these has been associated with decreased risk of developing chronic diseases (31, 33). It has been established that a higher intake of soluble fibers such as oats and chia-seeds has proven to decrease low-lipoprotein cholesterol (LDL-c) levels (34).

Meat contains several important fatty acids that are also essential for the body's functioning. Most commonly found in meat are saturated fatty-acids (SFA) and monounsaturated fatty-acids (MUFA)(35). Arachidonic acid(20:4n-6) and oleic acid(18:1*cis*-9) are two known primary examples of important fatty-acids found in meat (35). Arachidonic acid(20:4n-6) have an important role in the central nervous system of new-born and make up a great part of the brains phospholipids, and oleic acid(18:1 *cis*-9) has been proven to have beneficial effects on insulin sensitivity for further risk of the development of T2DM (27, 36-38). Dietary guidelines have over several years recommended avoiding over-consumption of these fatty acids to reduce the risk of disease, and correlation between T2DM and high consumption of animal fat has been studied. Studies found that consuming higher levels of animal fat than recommended by the

dietary guidelines, increases insulin sensitivity rather than having a beneficial effect (27, 39). Besides being a good source of nutrients, the bio-availability of these nutrients is higher in meat than in most plant-based foods, making meat essential in human diet (40-42).

While meat is rich in essential nutrients, overconsumption has proven to have some negative effects on human health. Studies show that meat, especially red and processed meat is linked to CVD, T2DM, and several types of cancer in both men and women (43, 44). It has been scientifically proven that red and processed meat is directly linked to colorectal cancer as well as complex diseases such as T2DM and CVD, and WHO and the International Agency for Research on Cancer (IARC) have classified processed meats as carcinogenic (4, 43, 45-47). Meat is rich in SFA which increases low-density lipoprotein cholesterol (LDL-c), and high intake of LDL-c increases blood cholesterol levels (35, 48). A review of epidemiological studies on health risks associated with meat consumption found that consuming meat, especially red and processed, is associated with higher mortality rate (43). It is evident that although meat is a good source of nutrients with high biological value, it also plays a central role in the epidemiology of chronic diseases as dietary cholesterol is mostly found in meat and eggs resulting in elevated s-cholesterol levels (27, 30). Therefore, understanding of the impact reducing meat consumption on the development of chronic diseases, such as T2DM, is essential.

1.3 Diabetes Mellitus

Diabetes mellitus is a group of several metabolic disorders where the production or use of insulin is insufficient, resulting in hyperglycemia (49). This is a condition where blood glucose is unable to enter the cells, either by lacking insulin-mediated transport due to low insulin production, or the body's ability to utilize the insulin itself (50). This usually occurs due to the pancreas' inability to produce insulin, or the body's ability to take up the insulin that is being produced and transport glucose into the cytosol of the cell (50, 51). It is estimated that 462 million people were living with T2DM in 2017, and the number is still rising (1). The number of people affected by diabetes was estimated to be 537 million people in 2021 and is expected to rise to around 645 million by 2030. This number is expected to be as high as 800 million in 2040 (52).

1.3.1 Type 1 Diabetes Mellitus

Estimates from 2020 show that type 1 diabetes account for 10% of all people affected by diabetes (53). Contrary to T2DM, type 1 diabetes is an autoimmune disease (54). The healthy cells in the pancreas that produce insulin, beta cells in the Islets of Langerhans, are attacked by the body's immune system – causing the production of insulin to decrease (55). People with type 1 diabetes have very little to no insulin production, causing their blood glucose levels to rise (56). Insulin is an important transporter of glucose in the blood, by unlocking the glucose channels in our body's cells, and thus making the glucose available for the cells to use (57). Without this mechanism, the glucose would not be able to be transported into the cell efficiently (58-60). However, though it is poorly understood, it has been reported that lifestyle factors, including diet, may have an impact on type 1 diabetes. Indeed, recent studies have found that correlation between obesity and type 1 diabetes, and it is most likely due to elevated serum cholesterol (s-cholesterol) buildup in the vessels (61). But more advanced clinical and biochemical investigations are needed to understand the interplay between lifestyle and development of type 1 diabetes.

1.3.2 Type 2 Diabetes Mellitus

The prevalence of T2DM continues to increase and is becoming a major concern globally, and it is ranked as the ninth leading cause of death (1). However, the prevalence of diabetes does not occur accidentally. Countries with high socio-economic development tend to have more cases of T2DM, and this is especially true in high income countries such as western-Europe, the USA, and other emerging economy countries like China and India (1). Although diabetes is seen to be increasing in well-developed countries, more studies are indicating that diabetes is rising in low-income countries as well (1, 62). Contrary to type 1 diabetes, environmental or lifestyle factors, such as diet and lack of physical activity, are the primary risk factors for T2DM (63). Some population groups seem to be affected more than others, with age and ethnicity being two driving factors (64). In terms of age, multiple studies found that elderly people have a higher risk of developing T2DM. Insulin resistance increases with age, and it is mainly because of factors like less muscle mass due to decreased physical activity, and less bone density (65, 66). T2DM is commonly diagnosed using Hemoglobin-A1c (HbA1c), which is a biomarker directly linked with blood glucose levels in the body over a longer time. It has been

proven a correlation between a higher meat and increasing HbA1c levels, making it a direct risk of development of T2DM (67).

1.4 Cardiovascular disease

Cardiovascular disease refers to a group of disease conditions occurring in the heart and blood vessels in the body. The diseases affect the blood flow in the vessels, contributing to heart diseases and stroke (68). There are different sub-groups within the disease, most of them affecting the heart and its arteries – but it also includes deep vein thrombosis and embolism in the lungs and brain. The latter two are often acute and difficult to detect before a blood clotting has been formed in the vein, and can be fatal if reaching the heart, lungs, or brain. Most CVDs are caused by a buildup of plaque and fats in the blood vessels, causing a weaker blood flow (69). Evidence has shown that poor diet and sedentary lifestyle are the most common risk factors for CVD (9, 70). In 2019 it was estimated that 523 million people worldwide were affected by CVD, and approximately 18 million people die of CVD annually (71).

1.5 Biomarkers

Biomarkers are important measurable indicators and structures in our bodies (72). These can vary from biochemical structures, to cellular and pathological alterations which functions as an indicator for health status or potential underlying disease, and are highly important to determine progression, diagnosis, prediction, and cause of disease (73, 74).

Within nutrition, biomarkers of food intake are used to determine the body's ability to absorb and utilize the nutrients, and it is an important tool to use when looking at the association between diet and health, and the markers reflect the metabolism of the consumed nutrients and the prevalence of possible disease (75-78). Measuring biomarkers as an indication of nutritional status alone may not give a full indication of disease, however, looking at nutritional status along with biomarkers of disease, strengthens the interpretation of occurrence of disease. Biomarkers directly linked to disease and health status are preferably used for a clinical assessment of disease, for example measurement of total s-cholesterol or LDL-c to determine CVD (76). Biomarkers such as HbA1c, LDL-c, high-density lipoprotein cholesterol (HDL-c), and total cholesterol (TC) are dominant biological markers within the body that are especially related and connected to T2DM and CVD.

1.5.1 HbA1c

Hemoglobin-A1c (HbA1c) is the most common biomarker used to evaluate and diagnose if an individual has T2DM. This is the so-called "long-term" blood glucose level, and it indicates the level of glucose over several months (79). For decades, diabetes has been diagnosed with fasting plasma glucose (FPG), and by oral glucose tolerance test (OGTT). However, studies have shown that long-term clinical parameters, such as HbA1c, is far more reliable and steady than short-term measurements (80). Additionally, HbA1c is specific, by measuring the average level of blood glucose over a longer time – in contrast to measuring post-prandial blood glucose which is affected by short-term food, fluids intake, stress, and exercise (81-84).

Glycated hemoglobin, HbA1c, consists of hemoglobin (Hb) with a glucose molecule attached to it (85). Hemoglobin is present in erythrocytes (red blood cells) making it possible for the cell to carry oxygen to the tissue (86). The formation of HbA1c is through structural change including a glucose molecule, and hemoglobin. A glucose molecule attaches to the N-terminal on the hemoglobin, forming a structure named Schiff's base. Once rearrangements has taken place, the structure changes to a glycated hemoglobin (HbA1c) (87). The glucose molecule is believed to stay attached until the erythrocyte is broken down, for a period of approximately 106-120 days, and this makes it possible to measure glycated hemoglobin over a longer period (88). The lifespan of erythrocytes varies between individuals, and because of this, measuring HbA1c levels as a specific number would not give the correct measurement. As result, it is commonly reported in percentages from 5.7% and up – where 5.7% is within the normal range. Levels above 5.7% are described as pre-diabetic, and diabetic above 6.4%. In other words, the HbA1c is a measurement of how much percentage of glucose is attached to the erythrocytes (80, 88, 89).

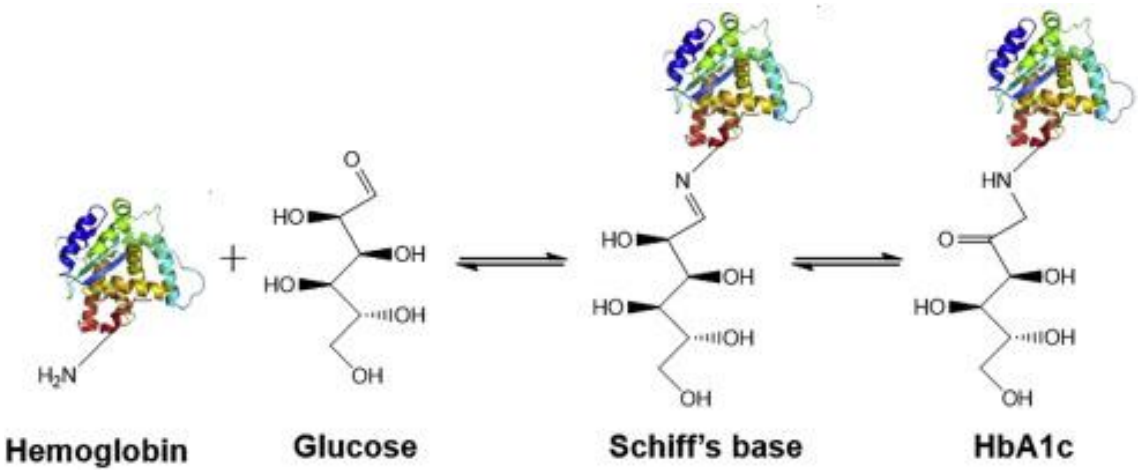

Figure 1: *Illustration of glycation process from hemoglobin, to HbA1c. A glucose molecule attaches to hemoglobin and forms a structure named Schiff's base. This structure is reversible, but once shifted onto the form of HbA1c, the glucose stays attached until the erythrocyte is broken down. Adapted from (Kaur et al., 2019) (87).*

1.5.2 LDL-cholesterol, HDL-cholesterol, and total cholesterol

The lipid known as cholesterol in our body is carried by two major types of lipoproteins, known as HDL-cholesterol (HDL-c) and LDL-cholesterol (LDL-c) (90), and the main task of these lipoproteins is to transport cholesterol through the blood system and to the liver for excretion (68). Cholesterol is first packed into lipoproteins before further transport due to it being a fat-soluble substance (91). The ratio between HDL-c and LDL-c in our blood must be optimal, meaning higher levels of HDL-c and lower levels of LDL-c, to avoid heart disease and stroke caused by the build-up of plaque (92). LDL-c is considered to be the "bad cholesterol" in the body, as it can increase the risk of developing CVD (92). Cholesterol transported by LDLs causes buildup of plaque and lipids in the arteries, which can lead to fatal blood clots (92, 93). Healthy and optimal levels of LDL-c are approximately 100 mg/dl, and the borderline to unhealthy levels are 130-160 mg/dl (94). These numbers are affected by numerous factors such as diet, weight, and physical activity, and these three modifiable risk factors are the most important ones. However, other conditions such as diabetes, gender, and genetics have also been assumed to have an impact on s-cholesterol levels (92).

Contrary to LDL-c, HDL-c is more commonly known as “the good” and heart-friendly cholesterol since it contributes to excretion of bad cholesterol. HDL-c transports cholesterol to the liver for excretion, making it a key component in the handling of cholesterol. The desirable levels of HDL-c are 1.6 mmol/L for all genders, though there is a slight variation in the lower levels. The reference value for men is set to be < 1 mmol/L and < 1.3 mmol/L for women (92, 95). Norwegian Health Informatics (NHI) recommends lowering intake of saturated fats in the diet which tends to raise LDL-c levels and replace it with unsaturated and polyunsaturated fats. Plant oils, whole grains rich in fiber, vegetables, omega-3 fatty-acids, and legumes are some recommended dietary sources to reduce LDL-c levels and maintain steady HDL-c levels (5, 96). These recommendations align with the increasing advocacy to shift from a meat-rich diet to a predominantly plant-based diet.

1.6 Justification

Detecting and looking at biomarkers early on can be crucial to prevent the development of diseases. CVD is a leading cause of mortality worldwide, and we know that the presence of diabetes increases the prevalence of CVD. Researchers have found a link between elevated blood sugar and hypertension, and several studies have discussed the mechanism and damage that glucose have on the blood vessels (97, 98). It is of importance to acknowledge the cardiovascular risk factor in patients with T2DM, diabetic dyslipidemia – where elevated LDL-c and low levels of HDL-c are the characteristics (99). Meat has been a prominent part of our society mostly due to its nutritional composition, but also because of the cultural and traditional value that meat holds globally. Thus, it is of importance to study the impact meat consumption and reduction has on relevant biomarkers that are directly linked to T2DM and CVD, at a time where these NCDs are the leading causes of mortality worldwide. However, when humans are reducing one type of food, it will have to be substituted by other foods in cases where the total energy intake remains stable. This is understood as the food substitution effect (100). In the case of meat reduction, there is some uncertainty related to the effect of that replacement. Theoretically, although meat is found associated with T2DM and CVD, it could also be possible that meat replacements are higher in starch and sugars, and increasing blood glucose levels. Therefore, it is of interest to evaluate the effect on biomarkers both for T2DM and CVD from the same pool of research as the systematic review and meta-analysis allow. The focus needs to be shifted towards overall meat consumption, and how this affects sustainability as much as

general health. Agriculture alone is responsible for a third of the anthropogenic greenhouse gas emissions, and a richer economy results in more consumption of meat (2). However, reducing meat could not only contribute to a positive outcome on health status by lowering s-cholesterol and blood glucose levels, but also reduces the risks of developing colorectal cancer and other cancer types as well (101). Thus, the effect on lowering LDL-c levels in T2DM patients, has been associated with a reduced risk of developing CVD (99).

2.0 Objectives

The aim of this systematic review and meta-analysis was to evaluate the effect of meat reduction on biomarkers of CVD and T2DM in adults.

Specific objectives of this review were as follows:

1. To investigate RCTs that reported on the effect of any meat reduction using biomarkers for CVD and T2DM as outcome measures, and do a quality assessment of these studies
2. To present a meta-analysis on the effect on LDL-cholesterol from any meat reduction
3. To present a meta-analysis on the effect on HDL-cholesterol from any meat reduction
4. To present a meta-analysis on the effect on HbA1c from any meat reduction
5. To present sub-group analysis of meta-analysis (secondary objective 2-4) on; study duration, diabetes status, and age-group.

3.0 Methodology

A systematic review and meta-analysis were conducted to evaluate the effect of meat reduction on nutritional biomarkers of sugars and lipids. This review is reported based on the Preferred Reporting Item for Systematic Reviews and Meta-Analyses (PRISMA) (102).

3.1 Eligibility Criteria

Studies were included in this review if they fulfilled the criteria set for population, intervention, comparison, outcome, and time frame (PICO(T)). The main criteria for PICO(T) are presented in **Table 1** and discussed in detail in subsequent sections.

Table 1: *Table of eligibility criteria. Studies must fulfill the criteria to be included.*

Population	Intervention	Comparison	Outcomes	Time frame
Adults ≥ 18 years, no limitation on sex	Meat reduction, substitution, or exclusion	Standard meat intake, habitual diet	HbA1c, HDL-c, and LDL-c	≥ 4 weeks of intervention time

3.1.1 Population

This systematic review targeted adults ≥18 years old, any sex or self-reported gender were included. The age limit was set to mainly include people who are able to give informed written consent on their own behalf, and the age-limit reflects that in most countries. This review included studies with both diabetic and non-diabetic participants.

3.1.2 Intervention

Randomized controlled trials were eligible for this review if they contained information on an intervention that reduced or excluded meat from the diet. However, studies in which meat was reduced or excluded but replaced with fish or seafood, were not eligible for this study in case this could affect the outcomes.

3.1.3 Comparison

A control diet was considered to be a habitual or standard diet in which meat products have not been reduced or excluded. It was considered important that the control group consumed a

habitual diet throughout the study, and that they consumed a higher amount of meat than the intervention group.

3.1.4 Outcome measures

The outcomes of interest for this review included HbA1c as a proxy of blood glucose, and HDL-c and LDL-c for lipids. The lipid biomarkers are directly linked with a high risk of cardiovascular disease, and it is well known that decreased levels of HDL-c and increased levels of LDL-c are associated with a higher risk of developing T2DM (103). Therefore, understanding the effect of meat reduction on these outcomes may play a role in preventing T2DM and CVDs.

3.1.5 Time frame

Trials were included in the review if they had a minimum duration of 4-weeks of intervention as that was considered appropriate in order to be able to detect any potential changes in the biomarkers. The HbA1c biomarker is present for 8-12 weeks and stored in the erythrocytes until the cell is broken down, approximately after 120 days (89). There is proven to be minor reduction on the lipid biomarkers after 4 weeks (104), but hopefully studies with longer duration will show greater effect on the outcomes. There was no upper limit on the duration of the study, making RCTs with long term follow-up (years) eligible.

3.1.6 Types of studies to be included

Only randomized controlled trials (RCT) were eligible for this review. This decision was made as RCTs are ranked higher when looking at the hierarchy of evidence (105). Well-designed RCTs should reduce the influence of socio-economic patterns which observational studies are more affected by. For example, people choosing a particular low-meat diet may very well have other characteristics in terms of lifestyle which may influence the outcomes of interest. Studies had to be available for full-text reading to be included for data extraction.

3.2 Databases and literature search strategy

A literature search was performed in PubMed, Embase, and Cochrane Library between the 10th and 12th of January 2022. A thorough search string was developed based on the eligibility criteria presented in **Table 1**. An overview of the literature search process in different databases

is presented in **Appendix 1**, **2**, and **3**. Briefly, our string included keywords with three Boolean operators; including (AND), (OR), or (NOT) to get a detailed and specific search. Studies were selected if the following criteria were met;

1. Prospective study design
2. Participants had to be divided into control or intervention group by random allocation, and preferably full concealing of allocation.
3. Availability for full-text reading
4. The studies must report data on at least one of the outcomes of interest
5. Studies must include clear description of the intervention (reduction, exclusion, or substitution of meat products from the diet).
6. Studies must have parallel arms

3.3 Study selection and data extraction

Literature from all databases was exported to EndNote 20 for duplicate removal. After removing duplicates, all records were exported in to *Rayyan screening tool* for title and abstract screening against eligibility criteria (106). Title and abstract screening were done by one review author, Marthe Synnøve Fiskaa Mila (MSFM). For articles where decisions could not be easily made, the review author discussed the issue with review supervisors. Screening of title and abstract was conducted in January and February of 2022, and was followed by full-text reading and data extraction from the eligible studies. Data were extracted using an Excel spreadsheet, and data was extracted from both baseline (pre-study) and end-line (end of study). Following information was extracted in both intervention group and control group: authors, title, country, year of publication, number of participants in both intervention and control group, gender, age, study duration, if participants were diabetic at baseline or not, type of intervention, energy restriction, type of substitute (soybeans or legumes), degree of substitution (meat, or meat + dairy), study design, and study outcomes (mean and standard deviation (SD) from both baseline and end-line). The preferred reporting item for systematic reviews and meta-analyses (PRISMA) was followed using the flow chart presented as **Figure 2** (102).

3.4 Statistical analysis

The analysis of extracted data was performed using STATA (version 17.0) and Review Manager software (RevMan version 5.4.1) (107, 108). For each outcome, effect size was summarized using random effect model meta-analysis and are presented in forest plots. We extracted data from baseline and end-line in both intervention and control-groups. Then after, the difference between baseline and end-line was calculated and used in the meta-analysis. Brief, mean differences were calculated by subtracting end-line data from baseline data (109).

A majority of the studies had reported data on mean and SD, making no other calculation than $SD_{difference}$ necessary. One study which contained multiple arms (110) reported data as standard error (SE), and SD had to be computed using RevMan software (108). Likewise, in studies where data were reported as median and inter-quartile range (IQR), mean and SD were calculated. Equations used for converting IQR and median to SD and mean are presented below as **Equation 2**, **3,** and **4** – and are published by Wan *et al.,* (2014) (111). $SD_{difference}$ had to be calculated before meta-analyses, and **Equation 4** was utilized for calculating this (109). The correlation, r, between baseline and end-line in **Equation 4**, was assumed to be 0.5.

$$I^2 = \frac{100\% * (Q - df)}{Q}$$

Equation 1: *Cochrane Reviews' I^2 equation for assessment of consistency among studies in a meta-analysis. Q represent Cochran's heterogeneity, which is calculated by summarizing squared deviation $\Sigma(x\text{-}x)^2$ from all studies in the analysis. df=degree of freedom. The equation was retrieved from Higgins et al., (2003) (112).*

$$S = \frac{q^3 - q^1}{1.35}$$

Equation 2: *Equation was used to calculate SD where data were reported as IQR, where q^1 and q^3 represent lower and upper quartile. 1.35 is a constant when the number of participants is $n \geq 50$. The equation was retrieved from Wan et al., (2014) (111).*

$$X = \frac{q^1 + m + q^3}{3}$$

Equation 3: *Equation used to calculate mean where data was reported as IQR. $q^1$and q^3 represent lower and upper quartile, and m is median. 3 is a constant when number of participants n ≥ 50. The equation was retrieved from Wan et al., (2014) (111).*

$$X = \frac{a + 2m + b}{4}$$

Equation 4: *Equation used to calculate mean where data was reported as median, where a(minimum value) + 2*m(median) + b(maximum value), and 4 is a constant. The equation was retrieved from Wan et al., (2014) (111).*

$$SD_{difference} = \sqrt{\left(SD_{baseline}{}^2 + SD_{endline}{}^2\right)} - (2 * r * SD_{baseline} * SD_{endline})$$

Equation 5: *Equation used to calculate standard deviation ($SD_{difference}$) of the difference between baseline and end-line on each outcome. Correlation, r, was assumed to be 0.5. The equation was retrieved from Vogtschmidt et al., (2021) (109).*

The results from the meta-analysis are presented as mean difference (MD) with a 95% confidence interval (CI) and with an alpha level at 0.05. We used I^2 statistics (**Equation 1**) to evaluate the heterogeneity and consistency among the studies (112).

3.5 Sub-group analysis

Sub-group analyses were performed to evaluate the impact of different variables on study outcomes. Specifically, sub-group analyses were performed on three variables:

1. Study duration (long duration ≥ 24 weeks, or short duration < 24 weeks)

2. Diabetes status (diabetic at baseline, or non-diabetic at baseline)
3. Age-group (adults aged ≥ 55 years, and adults aged < 55 years)

3.6 Quality assessment of eligible studies

In systematic reviews and meta-analyses, quality assessment of reviewed studies is of importance to reduce the risk of bias (RoB). Quality assessment on the included studies was performed by the review author using the *Cochrane Handbook for Systematic Review of Interventions* RoB tool by The Cochrane Collaboration (113, 114). RoB assessment encompassed evaluation of five domains which determined the quality of RCTs, including:

1. Concealing of allocation sequence
2. Non-randomization
3. Appropriate measurement of outcomes, blinding of personnel and assessors
4. Appropriate analysis tools
5. Appropriate selection of the reported results

Studies were classified as low, moderate, or high risk of bias. The optimal outcome is low to moderate RoB, meaning that the RCTs have been conducted and carried out with minimal impact. RoB was performed on all outcomes separately (LDL-c and HDL-c vs HbA1c). LDL-c and HDL-c uses the same method of analysis and is measured in serum, while HbA1c is measured in full-blood.

4.0 Results

Results are presented in a systematic layout. First presented are the literature searches in the databases, and the study selection process. Lastly, results of meta-analysis and sub-group analyses are presented subsequently.

4.1 Results of study selection

A total of 10 studies fulfilled eligibility criteria for this review. The study selection process presented in the PRISMA flow chart below (**Figure 2**). First, the search string was created and was ran in selected databases; PubMed, Embase, and Cochrane Library. Our literature search generated 3.658 records in total, from all databases. Removal of duplicated studies resulted in 2.907 studies ready for manual screening by review author Marthe Synnøve Fiskaa Mila (MSFM). After title and abstract reading, 139 records were left for full-text retrieval. 62 records were retrieved and further assessed for eligibility. Only 31 articles were available for full-text reading and eligibility assessment - leaving a total of 10 studies eligible for data extraction in this review. 21 studies were not eligible for this review, and an overview is presented in **Table 2**. Out of the 21 non-eligible studies, five studies were presented with the wrong intervention, and 10 studies had the wrong study design. Six studies did not report data on the outcomes of interest for this review.

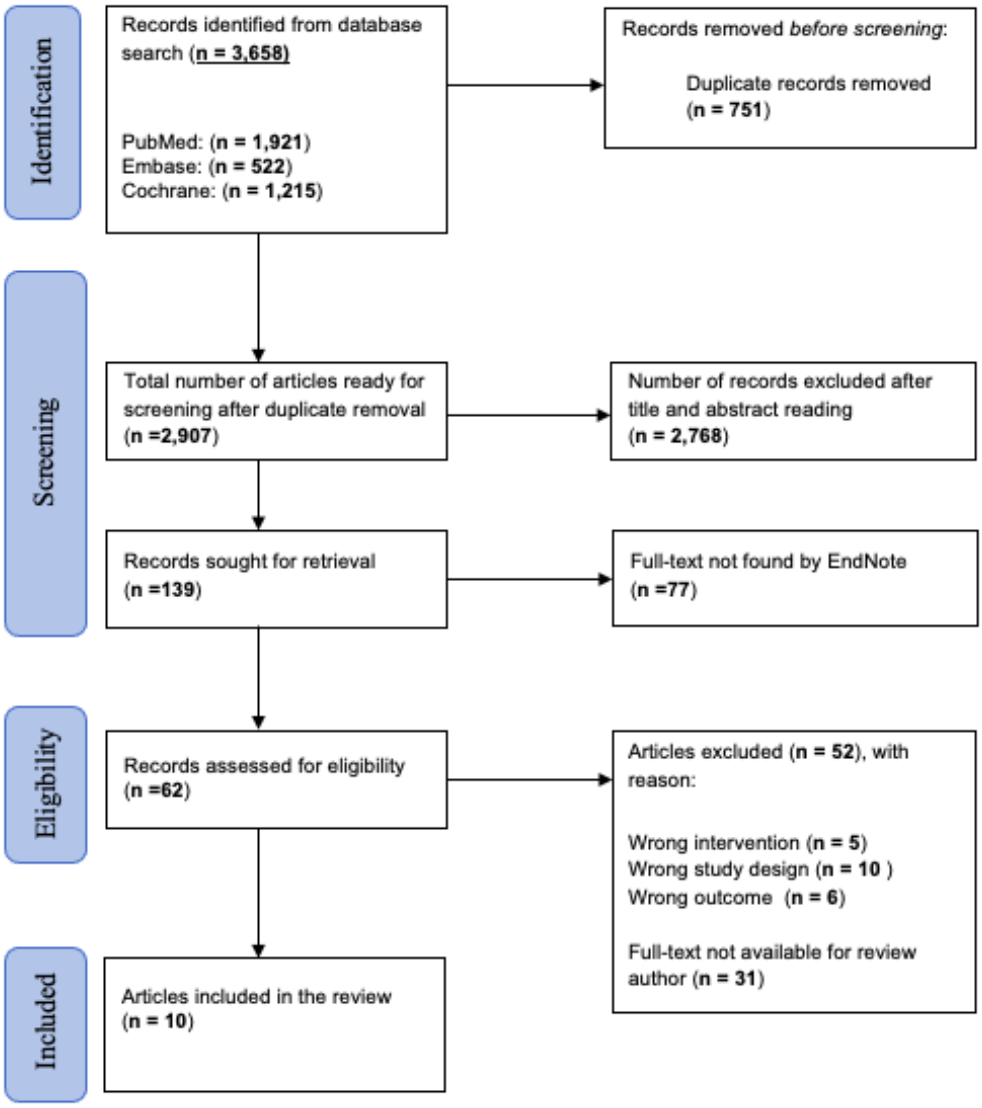

Figure 2: *PRISMA flow chart of study selection process. Records were first identified in the databases, then screened manually by the review author. 62 records were assessed for eligibility, and a total of 10 records were included in this review.*

4.2 Characteristics of the included studies

Characteristics of the included studies are presented in **Table 3,** and an overview of difference between baseline data and end-line data results on all outcomes is presented in **Table 4.** There were a total of 1.136 participants in the included studies, where 647 participated in the intervention groups and 665 participants in the control groups. 186 of the participants were male, and 559 were female. The included studies in the review were conducted from 2006-2021. Of the 10 included studies, all reported data on lipid biomarkers, and seven studies reported data on all outcomes of interest. Four of the studies had some form of energy restriction of approximately -500 kcal per day (115-118). Three of the four studies in which had energy restriction were only exclusive to the control group and not the intervention group (115, 116, 118), while one study reported on energy restriction in both groups (117). The remaining six out of the 10 eligible studies followed an *ad libitum* diet, meaning no restriction on calorie intake. Seven studies included participants with diabetes at baseline (115-121), and the remaining three studies included non-diabetic participants (110, 122, 123).

4.3 Intervention on LDL-c

A total of 1.136 participants from all 10 RCTs contributed data to a meta-analysis of LDL-c, presented as **Figure 3**. The results show that consumption of meat-reduced diets significantly decreased LDL-c compared to meat-rich diets (MD: -6.4 mg/dl, 95% CI: -10.8, -1.9). There was evidence of substantial heterogeneity in the effect between studies (I^2=63.4%).

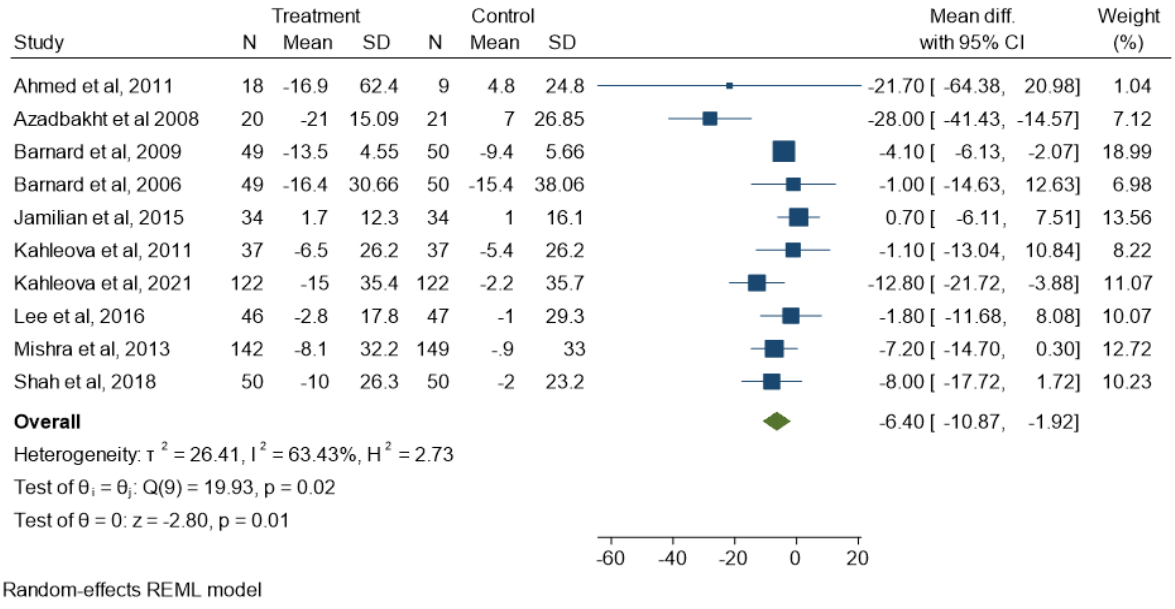

Figure 3: *Effect of reducing meat consumption on LDL-c. Meta-analysis performed on 10 studies showed a statistical significant reduction on LDL-c levels when participants reduced meat intake.*

Results from sub-group analyses on LDL-c are presented in **Appendix 4**, **5**, and **6**. There was evidence of significance between sub-groups on study duration (long- vs. short-duration), and studies of shorter duration had evidently lower LDL-c levels (MD: -5.25 mg/dl, 95% CI: -9.70, -0.79) (**Appendix 4**). Heterogeneity in the effect size was higher in long-duration studies (I^2=88.53%) than short-term studies (I^2=29.31%).

Similarly, there was not a significant effect on LDL-c between studies which had diabetic participants (MD: -5.20 mg/dl, 95% CI: 10.78, 0.39). There was, however, a significant effect in studies with non-diabetic participants at baseline (MD: -10.86 mg/dl, 95% CI: -17.36, -4.37) (**Appendix 5**). Heterogeneity was higher among studies which recruited participants with diabetes (I^2=72.54%) at baseline than in studies with participants who were non-diabetic (I^2=0%).

Likewise, there was no significant effect on the LDL-c outcomes in participants who were in the age-group < 55 years (MD: -3.09 mg/dl, 95% CI: -10.82, 4.65), and the heterogeneity was moderate to substantial (I^2=57.22%). However, there was evidence of a significant effect on LDL-c outcomes in the age-group ≥ 55 years (MD: -7.77 mg/dl, 95% CI: -13.62, -1.92) (**Appendix 6**). Heterogeneity was even more substantial in the studies including participants aged ≥ 55 years (I^2=66.21%).

4.4 Intervention on HDL-c

A total of 1.136 participants from all 10 RCTs contributed data to a meta-analysis of HDL-c, presented as **Figure 4.** From the results, there are no evidence of an impact of a meat-reduced diet on HDL-c compared to a meat-rich diet (MD: -0.65 mg/dl, 95% CI: -2.6, 1.3). There was evidence of substantial heterogeneity in the effect between studies (I^2=65.5%).

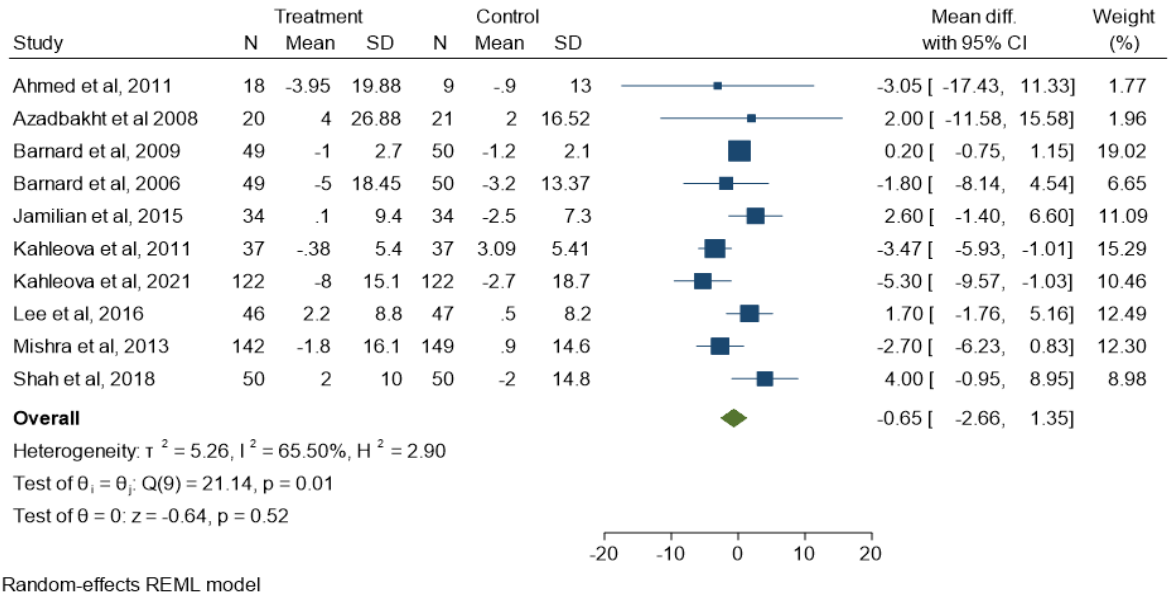

Figure 4: *Effect of reducing meat consumption on HDL-c. Meta-analysis on all 10 studies showed no statistical significant difference on HDL-c outcomes when participants reduced meat-intake.*

Results on sub-group analyses on HDL-c are presented in **Appendix 7, 8,** and **9.** 10 studies reported data on HDL-c, and there was no evidence of significant effect in either of the sub-groups on study duration (long duration: (MD: -1.25 mg/dl, 95% CI: -4.49, 2) and short duration: (MD: -0.33 mg/dl, 95% CI: -3.11, 2.45) (**Appendix 7**). Heterogeneity was slightly higher in the long-duration studies (I^2=73.79%), than in the studies of shorter duration (I^2=58.09%).

Likewise in the studies including participants with diabetes, there was no significant effect on HDL-c outcomes (MD: -0.53 mg/dl, 95% CI: -2.46, 1.39) (**Appendix 8**). Non-diabetic participants showed no evidence of significant effect on the outcomes as well (MD: -1.14 mg/dl, 95% CI: -8.05, 5.77). Heterogeneity was moderate in the studies with diabetic participants (I^2=59.1%), and substantial in the studies that included non-diabetic participants (I^2=71.33%).

No evidence of significant effect was found in the sub-groups on age. Both age-group < 55 years and age-group ≥ 55 years showed no significant impact of a meat-reduced diet on HDL-c (< 55 years: MD: -0.14 mg/dl, 95% CI: -5.33, 5.05 and ≥ 55 years: MD: -0.79 mg/dl, 95% CI:

-3.18, 1.59) (**Appendix 9**). Heterogeneity between studies in age-group < 55 years was high (I^2=73.63%), likewise in the age-group ≥ 55 years (I^2= 67.53%).

4.5 Intervention on HbA1c

A total of 1.000 participants from seven RCTs (115-118, 121-123) contributed with data to a meta-analysis of HbA1c, presented as **Figure 5.** The results show that consumption of meat-reduced diets decreased the levels of HbA1c compared to meat-rich diets (MD: -0.2 mg/dl, 95% CI: -0.3, -0.1). Heterogeneity in the effect size between studies was relatively low (I^2=32.87%).

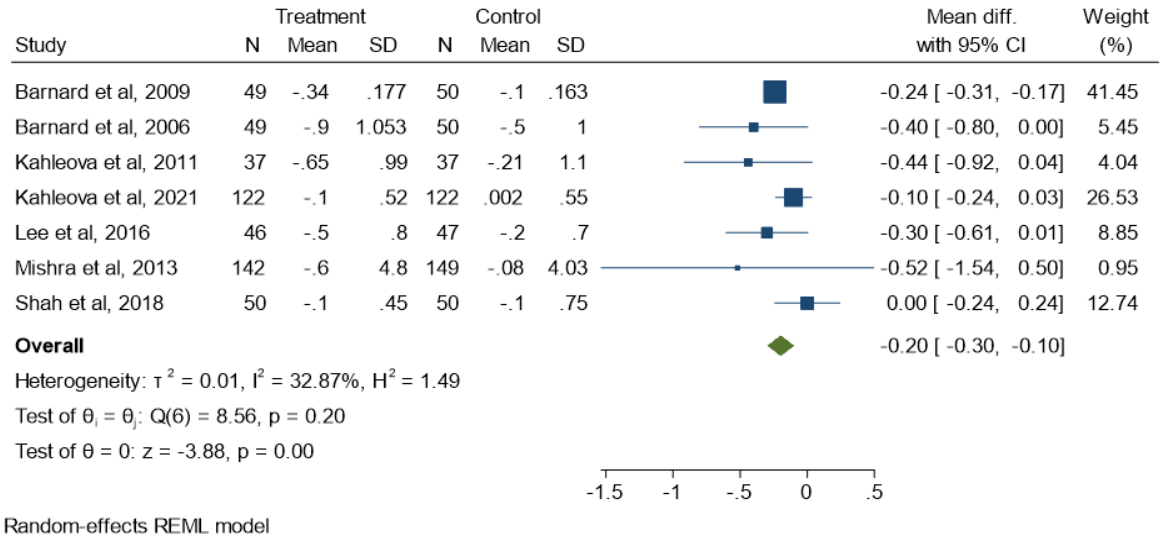

Figure 5: *Effect of reducing meat consumption on HbA1c. Meta-analysis from 7 studies showed a statistical significant reduction in HbA1c levels when participants reduced meat intake.*

Sub-group analyses on HbA1c are presented in **Appendix 10, 11,** and **12.** Results from sub-group analysis showed evidence of significant effect on HbA1c outcomes in studies of both long and short duration (**Appendix 10**). Heterogeneity in the longitudinal studies were low (I^2=0%), similar in the short-duration studies (I^2=6.57%).

In studies which participants were diabetic at baseline, there was a significant effect on the HbA1c levels when reducing meat (MD: -0.25 mg/dl, 95% CI: -0.32, -0.19). There was no significant effect on a meat reduced diet in non-diabetic participants when looking a HbA1c

outcomes (MD: -0.08 mg/dl, 95% CI: -0.20, 0.04) (**Appendix 11**). Heterogeneity was identical in both sub-groups (I^2=0%).

Likewise, there was no significant effect on HbA1c in the age-group < 55 years (MD: -0.52 mg/dl, 95% CI: -1.54, 0.50) Heterogeneity was not reported due to it only being one study. However, there was significant effect on the HbA1c outcomes in the age-group ≥ 55 years (MD: -0.19 mg/dl, 95% CI: -0.30, -0.09), and heterogeneity was low (I^2=37.36%) (**Appendix 12**).

4.6 Quality assessment of the studies

The results from RoB assessment are presented in Figure 6 and 7 for lipids outcomes (LDL-c and HDL-c) and HbA1c, respectively. In 10 studies which reported data on LDL-c and HDL-c, two studies (118, 121) were assessed as moderate risk of bias (some concerns), and 8 studies showed low risk of bias. An overview of RoB on LDL-c and HDL-c is presented in **Appendix 13**. Seven studies reported data on HbA1c. Of them, five were assessed as low risk of bias, and two studies (118, 121) were assessed as moderate RoB. RoB on studies reporting data on HbA1c is presented in **Appendix 14**.

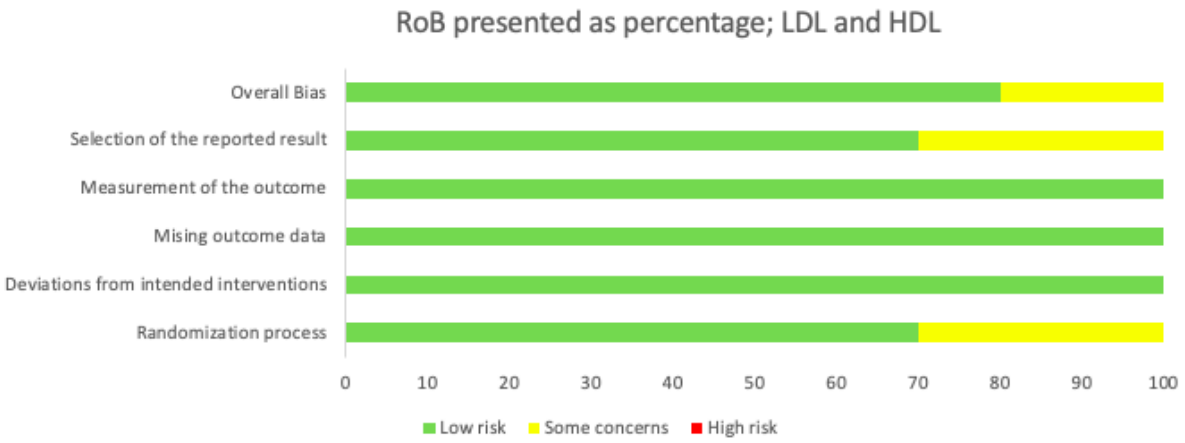

Figure 6: *Risk of Bias on 10 studies reporting data on LDL-c and HDL-c. 30% of the studies were assessed to have moderate risk of bias on the randomization process, and 30% of the studies were assessed as moderate risk of bias on the selection of the reported results process. 20% of overall bias was assessed to have moderate risk, some concern.*

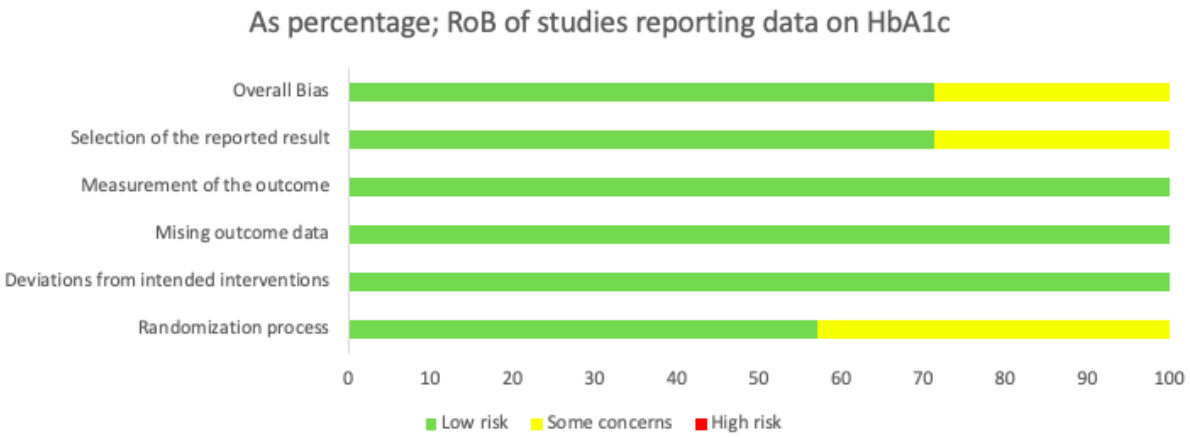

Figure 7: *Risk of Bias on 7 studies reporting data on HbA1c. 42.9% of the studies were assessed to have moderate risk of bias on the randomization process, and 28.6% of the studies were assessed to have moderate risk of bias on the selection of the reported results process. 28.6% of overall bias was assessed as moderate risk, some concern.*

5.0 Discussion

The aim of this study was to evaluate the effect of meat-reduced diets on biomarkers of T2DM and CVD in adults specified as HbA1c, LDL-c, and HDL-c, and perform quality assessment of the included studies. Meta-analysis on the effect on LDL-c, HDL-c, and HbA1c when reducing meat was performed. We also conducted sub-group analyses to investigate whether the effect differed depending on study duration, diabetes status, and age-group.

To recapitulate, 10 studies which included a total of 1.136 participants were eligible for this review. The main findings were that reduction of meat intake decreases the levels of LDL-c and HbA1c, but there was no evidence of a significant effect on the HDL-c levels. Sub-group analysis also showed a statistical significant effect on reduced LDL-c and HbA1c levels in studies of short duration, and in participants ≥ 55 years. In addition, we found evidence of reduced HbA1c levels in participants who were diabetic at baseline.

5.1 Evaluation of results

Diabetes and CVDs are ranked as top leading causes of death worldwide, and we can see a clear association between meat intake and mortality (124, 125). In the past five decades the consumption of meat has increased rapidly, and total meat intake per capita globally, is twice the average and recommended amount – and the intake is estimated to be rising (10, 13, 14, 16). While meat is of importance when looking at its nutritional status and in dietary culture, IARC and WHO have classified red and especially processed meat as a carcinogenic, making the increasing amount of consumption a major concern (45). Thus, one of the most important changes for reducing the risk of developing chronic diseases are dietary modifications, such as reduced animal protein intake (125, 126). At the same time, the number of people living with chronic diseases is as high as it has ever been, with 537 million people living with T2DM (2017) and approximately 523 million people with CVD in 2019 (52, 71). Evidence shows that consuming plant-protein is associated with reduction of TC and thus decreasing the risk of developing CVD and T2DM (127). A review of clinical studies on cholesterol-lowering effect of soy protein found that consuming less meat could positively affect those with hypercholesterolemia (extensive cholesterol levels), but had less effect in participants with normal s-cholesterol levels (128). Consuming plant-protein is known to be associated with

several cardio-metabolic benefits, such as lowered blood pressure, reduced risk of hypertension - which are a few precursors to CVD, and generally lowered TC levels (129). People who consume less meat tend to increase their dietary fiber intake, and several studies highlights the association between CVD and cardio-metabolic risk factors and the consumption of dietary fiber, where a higher intake was proven to reduce the risk and occurrence of disease (130, 131). A study published by Davies *et al.*, (1985) describes the pattern of fiber intake as meat intake was reduced in omnivores, vegetarians, and vegans – where omnivores consumed the least dietary fiber. The study found associations between high consumption of pulses, and lower fat intake in participants eating less meat (132). Evidently, vegetarians and vegans consume higher amounts of dietary fibers than meat-eaters, resulting in lower TC, which supports that vegetarians and vegans have lower chances of developing NCDs such as T2DM and CVD (132, 133). Having this in mind, huge parts of the world live in poverty and according to the Food and Agriculture Organization's most recent update, 2.3 billion people live with moderate and severe food insecurity and 828 million were experiencing hunger in 2021 (134). Thus, for people living in poverty, meat is considered an unaffordable luxury, and its reduction is not related to improvements in healthy diets rich in fiber, but rather unhealthy diets poor in nutrients and/or containing highly processed food rich in fats, starch, free sugars and salt. Therefore, food insecurity affects both under- and overnutrition. The latter could be labelled an 'obesity pandemic' giving high rises in NCDs such as CVD and T2DM (135, 136). Policies promoting meat reduction would also need to promote a healthy diet reaching all. This perspective needs to be communicated alongside all biomedical considerations on societal meat reduction. However, a larger food policy debate is considered outside the scope of this thesis.

5.1.1 Findings from sub-group analysis

Sub-group analysis was performed in three groups. We included study duration to explore whether the time frame of intervention would have an impact on the effect size. We then performed analysis based on whether the participants were diabetic at baseline or not, to see if a potential reduction in biomarkers would positively affect diabetic or non-diabetic participants more – and lastly, age of the participants were considered. Previous studies have found that most people living with T2DM are above the age of 50 years, and thus a comparison between specific sub-groups would establish even more who a meat-reduced diet is relevant for (65).

Our main findings from the sub-group analyses when looking at study duration showed varying results for both LDL-c and HbA1c levels. LDL-c levels were lower in studies of shorter duration (< 24 weeks), while levels of HbA1c were reportedly lower in studies of both short- and long-duration. In the studies where participants were diabetic at baseline, meat reduction was associated with lower levels of HbA1c. However, participants who were non-diabetic at baseline seemed to benefit from a meat-reduced diet too, as results showed a significant difference in LDL-c levels. In terms of age, reduction of meat intake had a positive effect on both LDL-c and HbA1c levels in adults aged ≥ 55 years. To summarize, a meat-reduced diet showed a statistical significant effect in reduction of both LDL-c and HbA1c levels, but it did not have an impact on HDL-c outcomes.

5.1.2 LDL-c

The meta-analysis performed on LDL-c data showed evidence of a statistical significant and clinical relevance in effect size when consuming a meat-reduced diet. As previously stated, LDL-c is also known as the "bad cholesterol", and the main reason for buildup of plaque in the arteries – potentially leading to CVDs and diabetic dyslipidemia (92, 99). Previous studies performed on this topic shows reduction of LDL-c (30, 137), and this systematic review and meta-analysis supported previous research. Our meta-analysis showed a substantial reduction in LDL-c levels in 9 out of 10 studies, with a MD: -6.4 mg/dl. Optimal levels of LDL-c are less than 100 mg/dl, whereas unhealthy levels are reaching 130-160 mg/dl and higher (94). A meta-analysis by Anderson *et al.,* (1995)(138) found an association between soybean consumption and reduction in serum lipids. The meta-analysis concluded with a 12.9% reduction in LDL-c levels, and 9.3% reduction of total cholesterol (137, 138), which is consistent with the results of our meta-analysis. Reduction in LDL-c levels is associated with lower risk of CVDs in patients with T2DM, and this was also seen in our analysis (99).

Our sub-group analysis on study duration indicated a reduction in LDL-c levels in studies of shorter duration. A one-month study on meat replaced with tofu by Ashton *et al.,* (2000) presented lower levels of LDL-c. Yokoyama *et al.,* (2017) (104) mentions a slight reduction in serum lipids after a period of just 4 weeks, supporting both the findings of Ashton *et al.*, (2000) (139), and the results from our sub-group analysis on a short intervention and effect.

From the sub-group analysis based on age group, we could see an effect of meat-reduction on LDL-c levels in adults ≥ 55 years compared to participants who were < 55 years. Our results are comparable to earlier findings by Abeysekara *et al.*, (2012) who found that consumption of a pulse-based diet was associated with reduction of LDL-c and TC in individuals above the age of 50. The study was conducted over a period of 8 weeks, similar to two studies in the sub-group analysis in this review (110, 122, 140). While this age group is at most risk for developing NCDs, not many previous studies have experimented on cardio-metabolic risk factors such as meat exclusively on this age group – and the study by Abeysekara *et al.*, (2012) is one of few. However, a recently conducted cohort study by Wang *et al.*, (2022) investigated the association between red meat intake and risk of cardio-metabolic diseases in adults above 65 years, and found that high red meat intake was associated with incidents of CVD but not specifically due to elevated s-cholesterol levels (141). Since the risk of developing T2DM and other NCDs increases with age, but more research on life-style and diet changes would benefit our understanding.

5.1.3 HDL-c

HDL-c, also known as the heart-friendly cholesterol in the body is an important biochemical parameter and lipid, and it is associated with cardio-metabolic benefits when levels are slightly raised. Desirable levels of HDL-c in both men and women are above 1.6 mmol/L (95). A study on soy protein as meat replacement indicated an overall positive effect on HDL-c levels when reducing meat and animal protein in the diet (138), and a fully plant-based diet containing no animal protein has proven to be of significance when looking at dietary treatment and prevention of NCDs such as CVD and diabetes (133). However, results from the meta-analysis in this review showed no evidence of an impact of a meat-reduced diet on the outcome. Lowered HDL-c levels are common in people with diabetic dyslipidemia, which can lead to a higher risk of CVD, especially in T2DM patients (99). Although studies show reduced HDL-c levels in those consuming a vegetarian or vegan diet, levels of HDL-c were still higher in meat-eaters (104, 142). We noted similar outcomes from our meta-analysis, where 60% of the included studies showed results of lowered HDL-c levels in the intervention groups. However, data from the meta-analysis were not of statistical significance, implying with the sampled and including population that the effect size was very small.

Sub-group analyses on HDL-c did also lack evidence of significance on the outcome. Based on the results, there was no effect on the HDL-c outcomes on a long-duration study where participants consumed meat-reduced diets compared to meat-rich diets. Likewise, looking at the data of the short-duration studies, there was no effect on the HDL-c outcomes in the intervention and control groups as well. As previously stated, we expected s-cholesterol to have a minor reduction in just 4 weeks of dietary change, and longer intervention time would possibly be more influential on the biomarkers (104). Considering the meta-analysis only included three long-duration studies, our analysis on HDL-c might now have been sufficiently powered to detect a statistical significant difference in the HDL-c marker. Our results should also be interpreted with caution due to this.

Seven studies reported data on HDL-c in participants who were diabetic at baseline and were included in the sub-group analysis performed on diabetes status. The results showed no statistical significant effect on HDL-c outcomes, when participants were diabetic pre-study and consuming a meat-reduced diet. Nonetheless, it might be relevant for future research to look at other factors such as environmental and life-style factors in comparison to their diabetes status. When looking at diabetic participants within the different studies, four of the seven studies were of short duration which could have an impact on the outcome due to short intervention time. Again, this could possibly be relevant for further research when studying the effect of meat-reduction, and who it benefits the most.

5.1.4 HbA1c

Our meta-analysis indicated that consumption of a meat-reduced diets significantly reduced HbA1c levels compared to a meat-rich diets. Heterogeneity between the included seven studies was low meaning little variation in effect size. Previous studies have shown that consuming meat-reduced diets, either vegetarian or vegan, has a positive effect on blood glucose levels (143, 144). HbA1c is measured in percentage of how much glucose is attached to the erythrocytes. Normal levels of HbA1c is measured to be 5.7%, where levels above 5.7% is assumed to be pre-diabetic, and diabetic above 6.4% (88, 89).

Looking at a few studies from our meta-analysis who reported data on HbA1c, Barnard *et al.,* (2009) (115) performed a 74-week clinical trial of a low-fat vegan diet vs a conventional diet

as treatment of T2DM. It was reported that medication use for T2DM was considerably reduced in 35% of the participants who consumed a low-fat vegan diet. In relation to one other study included in this review, Kahleova *et al.*, (2011) (117) reported that medication use for diabetes was reduced in 43% of the participants who consumed a vegetarian diet compared to the control group who consumed their habitual diets (5%).

Results from the meta-analysis showed evidence of reduced levels of HbA1c in both intervention and control groups, however, one study (123) showed results of elevated HbA1c levels in the control group. Looking at the results presented in **Table 4**, we can see that the differences between the intervention group and control group is somewhat substantial. HbA1c levels were slightly higher in the group consuming a meat-rich diet, than in the intervention group consuming a meat-reduced diet.

Age is a prominent factor when looking at who T2DM affects, and older adults are more prone to develop diabetes. Looking at the results from our meta-analysis, one can indicate that a meat-reduced diet would be beneficial for this age group. However, assessing HbA1c levels when looking at meat consumption solely in older adults are still lacking, and a full understanding of meat-reduction on protein and other micronutrients is currently debatable (145).

5.2 Evaluation of methodology

This systematic review followed approaches described in the *Cochrane Handbook for Systematic Review of Interventions* by The Cochrane Collaboration (114) to ensure a robust and systematic methodology and execution of the review. The objectives and scope of the review are stated, and multiple databases were searched with a pre-defined search string which was approved by review supervisor before the search took place. Eligibility and inclusion criteria of studies were thoroughly defined and described, with no limitation on language, sex, country, or year. However, clear exclusion criteria have not been stated. One review author, MSFM, screened and assessed the studies by using *Rayyan screening tool* (106), and two independent authors (review author and review supervisor) approved the included studies before data extraction.

Strengths in this review varies. Explicit methodology was performed, and a narrow literature search and research question was conducted. This is of importance to create a robust review. Search and selection of the studies to be included is highly comprehensive, making the review very specific. When assessing the study design used in this review (RCTs), meta-analysis performed on RCTs is preferred in contrary to observational studies. Observational studies tend to be more affected by socio-economic patterns, which can both increase the risk of bias, as well as influence the results of the intervention. There are also some limitation to the review. While multiple databases were searched for literature, we only performed literature search in three databases. Review author did not perform a hand-search, which is thought to be a critical part of a review. A broader search could have resulted in more eligible studies that would not have been found through standard database search. Although several databases were thoroughly searched systematically by review author MSFM with assistance from review supervisor, the chances of having missed important RCTs in the search process is possible.

The quality of the included studies is of importance, and bias was assessed by using the Cochrane risk of bias tool (113), which showed a general low risk of bias in the studies. However, bias can have occurred in the selection or publication process of the studies. Although review author assessed RoB, funnel plot was not performed to assess publication bias. When looking at quality, the review author focused on the five domains presented by the RoB tool:

1. Concealing of allocation sequence
2. Non-randomization
3. Appropriate measurement of outcomes, blinding of personnel and assessors
4. Appropriate analysis tools
5. Appropriate selection of the reported results

Although two studies (118, 121) were assessed with moderate risk of bias, there was little-to-no external influence on the included studies. None of the 10 studies deviated from the intended intervention, and neither of the studies indicated any RoB when looking at the analysis methods. Three studies (115, 116, 118) reported moderate RoB in the randomization process by algorithm results but was assessed as low risk by review author, since the concealing of allocation sequence was not relevant for randomization due to the assignments of group being done

simultaneously. Since RoB was performed on separate outcomes (lipids and glucose biomarkers), all studies were assessed as low RoB on the reporting of outcomes as well.

The majority of this review and meta-analysis was semi-computed by programs throughout the review period. Databases search (PubMed, Embase, and Cochrane Library), duplicate removal (EndNote), and statistical analysis (RevMan and STATA) were all checkpoints performed by computer tools, strengthening the reduction of miscalculations, missing literature, and poor literature selection for this review. Literature screening that was performed manually including full-text reading, and inclusion and exclusion assessment by review author could have had an impact on both analysis and further work. However, thorough eligibility screening of the included studies later on in the literature selection, and data extraction was done with high caution to avoid losing key-data and studies along the way.

The countries in which the included studies took place varied. One study was conducted in Brazil (110), one in the Czech Republic (117), one in Iran (120), and one in Korea (118). However, the remaining six studies were conducted in the USA (115, 116, 119, 121-123), meaning that there was little to no variation on countries and ethnicities included in 60% of the studies, and thus making the results and outcomes more relevant for western countries and their dietary eating patterns and occurrence of disease. Although some of the studies divided participants after ethnicity within the country the study was conducted in, other large parts of the world such as Africa, most of Europe, and Australia are not taken into consideration when we are looking at the results from our meta-analyses. Studies in this meta-analysis took place between 2006-2021. While the studies were relatively recently conducted, some key data from early studies may have changed over time – if compared to more recent studies. Another limitation of this review could be sample size of the included studies. While the number of participants were adequate and gave plausible results, studies with higher sample sizes would be of interest to look at in relation to the outcomes of this review.

It is of importance to highlight the findings of this review and meta-analysis, and that it could be of importance in regard to public health, and mortality in a time where CVD and T2DM are leading causes of death worldwide. Comparison of the outcome of our analyses in relation to similar studies and other reviews, makes it plausible and gives us reason to believe that our

findings are trustworthy. We can see consistency in the results from similar systematic reviews and cohort studies on how meat-reduced diets are associated with lowered levels of both LDL-c and HbA1c – which correlates with our findings in the meta-analysis.

5.3 Future perspectives

Further research would need to include more diversity on substitution diets and elaborate on meat types, and production issues. Grouping meat reduction without considering type of meat or agricultural issues is at best, oversimplification. A deeper understanding of other underlying conditions including anthropometry, blood pressure and other clinical parameters, and micronutrient status could be of interest as it would be of relevance to a larger share of the population – other than those affected by chronic disease. Meat reduction is in general implemented more and more, whether it is due to environmental factors or health-related reasons, and it would be of interest to get more general knowledge on the effect of meat-reduced diets covering wider perspectives.

More research on different dietary patterns in relation to glycemic control, as well as diabetes management and its association with insulin resistance and sensitivity in adults is important to establish. Health economic priorities needs to be established internationally including nutrition policy and nutritional education as a tools for prevention of chronic diseases. It could be a potential of using HbA1c as an early main indicator for T2DM, and thus with interventions prevent development of T2DM or CVD. However, the establishment of this as routine screening in combination with prevention and promotion of healthy diets need further scientific and health policy priorities.

6.0 Conclusion

The aim of this study was to observe the effect of a meat-reduced diet on biomarkers of T2DM and CVD in adults. Reduction of meat consumption indicates notable evidence of reduced LDL-c and HbA1c levels, but have no significant effect on HDL-c levels. These findings support the guidelines by WHO and NHI to consume less meat in relation to the increased risk of developing type 2 diabetes and cardiovascular disease (3-5). However, our results should be interpreted with caution since there was evidence of substantial heterogeneity in some of the outcomes.

7.0 References

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8.0 Figures and Tables

Table 2: Non-eligible studies. The studies were assessed for eligibility, but excluded due to the listed reasons.

Authors, year	Title	Reason
Alleman Jr. *et al.*, 2013 (146)	Both a traditional and modified Daniel Fast improve the cardio-metabolic profile in men and women	Wrong study design, no parallel arms
Bähr *et al*., 2013 (147)	Lupin protein positively affects plasma LDL cholesterol and LDL:HDL cholesterol ratio in hypercholesterolemic adults after four weeks of supplementation: a randomized, controlled crossover study	Wrong intervention
Barnard *et al.*, 2009 (148)	A low-fat vegan diet elicits greater macronutrient changes, but is comparable in adherence and acceptability, compared with a more conventional diabetes diet among individuals with type 2 diabetes	Wrong outcome measurements (FFQ)
Bergeron *et al.*, 2019 (149)	Effects of red meat, white meat, and non-meat protein sources on atherogenic lipoprotein measures in the context of low compared with high saturated fat intake: a randomized controlled trial	Wrong study design, crossover
Crimarco *et al.*, 2020 (150)	A randomized crossover trial on the effect of plant-based compared with animal-based meat on trimethylamine-N-oxide and cardiovascular disease risk factors in generally healthy adults: Study With Appetizing Plant food—Meat Eating Alternative Trial (SWAP-MEAT)	Wrong study design, no parallel arms
Djekic *et al.*, 2020 (151)	Effects of a Vegetarian Diet on Cardiometabolic Risk Factors, Gut Microbiota, and Plasma Metabolome in Subjects With Ischemic Heart Disease: A Randomized, Crossover Study	Wrong study design, no parallel arms

Hermansen *et al.*, 2001 (152)	Beneficial Effects of a Soy-Based Dietary Supplement on Lipid Levels and Cardiovascular Risk Markers in Type 2 Diabetic Subjects	Wrong study design, no parallel arms
Hilpert *et al.*, 2005 (153)	Lipid Response to a Low-Fat Diet with or without Soy Is Modified by C-Reactive Protein Status in Moderately Hypercholesterolemic Adults	Wrong study design, no parallel arms
Hosseinpour-Niazi *et al.*, 2015 (154)	Non-soya legume-based therapeutic lifestyle change diet reduces inflammatory status in diabetic patients: a randomized cross-over clinical trial	Wrong outcomes
Kahleova *et al.*, 2018 (155)	A plant-based diet in overweight individuals in a 16-week randomized clinical trial: metabolic benefits of plant protein	Wrong outcomes
Kitano-Okadaa *et al.*, 2019 (156)	Safety and efficacy of adzuki bean extract in subjects with moderate to high LDL-C: a randomized trial	Wrong intervention
Sofi *et al.*, 2018 (157)	Low-Calorie Vegetarian Versus Mediterranean Diets for Reducing Body Weight and Improving Cardiovascular Risk Profile	Wrong study design, no parallel arms
Teede *et al.*, 2001 (158)	Dietary Soy Has Both Beneficial and Potentially Adverse Cardiovascular Effects: A Placebo-Controlled Study in Men and Postmenopausal Women*	Wrong intervention
Teixeira *et al.*, 2000 (159)	Effects of feeding 4 levels of soy protein for 3 and 6 wk on blood lipids and apolipoproteins in moderately hypercholesterolemic men	Wrong intervention
Van Nielen *et al.*, 2014 (160)	Partly Replacing Meat Protein with Soy Protein Alters Insulin Resistance and Blood Lipids in Postmenopausal Women with Abdominal Obesity	Wrong study design, no parallel arms

Wheeler *et al.*, 2002 (161)	Animal Versus Plant Protein Meals in Individuals With Type 2 Diabetes and Microalbuminuria. Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men	Wrong study design, no parallel arms
Wong *et al.*, 1998 (162)	Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men	Wrong study design, no parallel arms
Barnard *et al.*, 2009 (163)	D2 Dopamine receptor Taq1A polymorphism, body weight, and dietary intake in type 2 diabetes	Wrong outcomes
Turner-McGrievy *et al.*, 2011 (164)	Decreases in Dietary Glycemic Index Are Related to Weight Loss among Individuals following Therapeutic Diets for Type 2 Diabetes	Wrong outcomes
Adamsson *et al.*, 2011 (165)	Effects of a healthy Nordic diet on cardiovascular risk factors in hypercholesterolaemic subjects: a randomized controlled trial (NORDIET)	Wrong intervention, no meat reduction
Burke *et al.*, 2007 (166)	Effects of a vegetarian diet and treatment preference on biochemical and dietary variables in overweight and obese adults: a randomized clinical trial	Wrong outcomes

Table 3: Included studies and their characteristics

Authors, year, reference	Country	Age-group	Participants, n	Duration	Diabetes status	Energy restrictions	Substituted
Ahmed *et al.*, 2011 (110)	Brazil	≥ 55 years	(n=27)	8 weeks	Non-diabetic	None	Meat, dairy
Azadbakht *et al.*, 2008 (119)	USA	≥ 55 years	(n=41)	4 years	Diabetic	None	Meat
Barnard *et al.*, 2009 (115)	USA	≥ 55 years	(n=99)	74 weeks	Diabetic	In control group	Meat, dairy
Barnard *et al.*, 2006 (116)	USA	≥ 55 years	(n=99)	22 weeks	Diabetic	In control group	Meat, dairy
Jamilian *et al.*, 2015 (120)	Iran	< 55 years	(n=68)	6 weeks	Diabetic	None	Meat
Kahleova *et al.*, 2011 (117)	Czech - Republic	≥ 55 years	(n=74)	24 weeks	Diabetic	In both groups	Meat
Kahleova *et al.*, 2021 (123)	USA	≥ 55 years	(n=244)	16 weeks	Non-diabetic	None	Meat, dairy
Lee *et al.*, 2016 (118)	Korea	≥ 55 years	(n=93)	12 weeks	Diabetic	In control group	Meat, dairy
Mishra *et al.*, 2013 (121)	USA	< 55 years	(n=291)	18 weeks	Diabetic	None	Meat, dairy
Shah *et al.*, 2018 (122)	USA	≥ 55 years	(n=100)	8 weeks	Non-diabetic	None	Meat, dairy

Table 4: Mean differences from data presented in the included studies (end-line subtracted from baseline data). **INT** represents intervention groups and **CONT** represents control groups. N/A = not applicable.

Authors, year	Diff_LDL_INT	Diff_LDL_CONT	Diff_HDL_INT	Diff_HDL_CONT	Diff_HbA1c_INT	Diff_HbA1c_CONT
Ahmed *et al.*, 2011	-16.9	4.8	-4.0	-0.9	N/A	N/A
Azadbakht *et al.*, 2008	-21	7.0	4.0	2.0	N/A	N/A
Barnard *et al.*, 2009	-13.5	-9.4	-1.0	-1.2	-0.34	-0.1
Barnard *et al.*, 2006	-16.4	-15.4	-5.0	-3.2	-0.9	-0.5
Jamilian *et al.*, 2015	1.7	1.0	0.1	-2.5	N/A	N/A
Kahleova *et al.*, 2011	-6.5	-5.4	-0.4	3.1	-0.65	-0.21
Kahleova *et al.*, 2021	-15	-2.2	-8.0	-2.7	-0.1	0.002
Lee *et al.*, 2016	-2.8	-1.0	2.2	0.5	-0.5	-0.2
Mishra *et al.*, 2013	-8.1	-0.9	-1.8	0.9	-0.6	-0.08
Shah *et al.*, 2018	-10	-2.0	2.0	-2.0	-0.1	-0.1

9.0 Appendices

Appendix 1: Database search: Search string in **PubMed**

Step	Query	Hits
1	((((((((((non-meat diet) OR (plant protein)) OR (meat reduc*)) OR ("sustainable diet")) OR (plant-based protein)) OR (meat replac*)) OR ("non-animal protein")) OR (meat alternatives)) OR (protein alternatives)) OR (vegetarian)) OR (meat analogues)) OR (vegan) AND ((clinicaltrial[Filter] OR randomizedcontrolledtrial[Filter]) AND (humans[Filter]) AND (alladult[Filter])) **Filters: Clinical Trial, Randomized Controlled Trial, Humans, Adult: 19+ years**	**7,595**
2	((((((("high meat intake") OR ("normal diet")) OR ("animal foods")) OR (omnivor*) OR (meat)) OR ("animal protein")) OR ("meat rich diet")) OR ("meat-based diet") AND ((clinicaltrial[Filter] OR randomizedcontrolledtrial[Filter]) AND (humans[Filter]) AND (alladult[Filter])) **Filters: Clinical**	**1,486**

Step	Query	Hits
	Trial, Randomized Controlled Trial, Humans, Adult: 19+ years	
3	((((((((((Glucose) OR (HbA1c)) OR (Hb1c)) OR (A1c)) OR (glycohemoglobin)) OR ("Diabetes Mellitus")) OR ("glycated hemoglobin")) OR ("serum glucose")) OR ("type 2 diabetes")) OR (T2DM))) OR ("glucose-biomarker*")) OR ("blood-sugar") OR (diabet*) AND ((clinicaltrial[Filter] OR randomizedcontrolledtrial[Filter]) AND (humans[Filter]) AND (alladult[Filter])) **Filters: Clinical** Trial, Randomized Controlled Trial, Humans, Adult: 19+ years	**53,588**
4	((((((((("lipid marker*") OR ("high density lipoprotein*")) OR (HDL)) OR ("low density lipoprotein*")) OR (LDL)) OR (hypercholesterolemia)) OR ("LDL-cholesterol")) OR ("HDL-cholesterol")) OR ("lipid biomarker*")) OR (triglyceride*)) **Filters: Clinical Trial, Randomized Controlled Trial, Humans, Adult: 19+ years**	**22,485**

Step	Query	Hits
5	(#1) OR (#2) **Filters: Clinical** Trial, Randomized Controlled Trial, Humans, Adult: 19+ years	**8,447**
6	(#1 OR #2) AND (#3) **Filters: Clinical Trial, Randomized Controlled Trial, Humans, Adult: 19+ years**	**1,210**
7	(#1 OR #2) AND (#4) **Filters: Clinical Trial,** Randomized Controlled Trial, Humans, Adult: 19+ years	**1,104**
8	((#1 OR #2) AND (#3)) OR ((#1 OR #2) AND (#4)) **Filters: Clinical Trial, Randomized Controlled Trial, Humans, Adult: 19+ years**	**1,921**
Total:		**1,921**

Appendix 2: Database search: Search string in **Embase**

Step	Query	Number of hits
1	(non-meat diet or plant protein or meat reduc* or "sustainable diet" or plant-based protein or meat replac* or "non-animal protein" or "meat alternatives" or "protein alternatives" or vegetarian or "meat analogues" or vegan).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	**29,565**
2	limit 1 to (human and (clinical trial or randomized controlled trial) and (adult <18 to 64 years> or aged <65+ years>))	**487**
3	("high meat intake" or "normal diet" or "animal foods" or omnivor* or meat or "animal protein" or "meat-rich diet" or "meat-based	**98,384**

Step	Query	Number of hits
	diet").mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	
4	limit 3 to (human and (clinical trial or randomized controlled trial) and (adult <18 to 64 years> or aged <65+ years>))	1,291
5	(glucose or HbA1c or Hb1c or A1c or glycohemoglobin or "Diabetes Mellitus" or "glycated hemoglobin" or "serum glucose" or "type-2-diabetes" or T2DM or "glucose biomarker*" or "blood-sugar" or diabet*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading	1,827,021

Step	Query	Number of hits
	word, floating subheading word, candidate term word]	
6	limit 5 to (human and (clinical trial or randomized controlled trial) and (adult <18 to 64 years> or aged <65+ years>))	65,674
7	("lipid marker*" or "high density lipoprotein*" or HDL or "low density lipoprotein*" or LDL or hypercholesterolemia or "LDL-cholesterol" or HDL-cholesterol or "lipid biomarker*" or triglyceride*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	450,376
8	limit 7 to (human and (clinical trial or randomized controlled trial) and (adult	26,099

Step	Query	Number of hits
	<18 to 64 years> or aged <65+ years>))	
9	1 or 3	**124,499**
10	(1 or 3) and 5	**9,886**
11	(1 or 3) and 7	**5,466**
12	10 or 11	**13,249**
13	limit 12 to (human and (clinical trial or randomized controlled trial) and (adult <18 to 64 years> or aged <65+ years>))	**522**
Total:		**522**

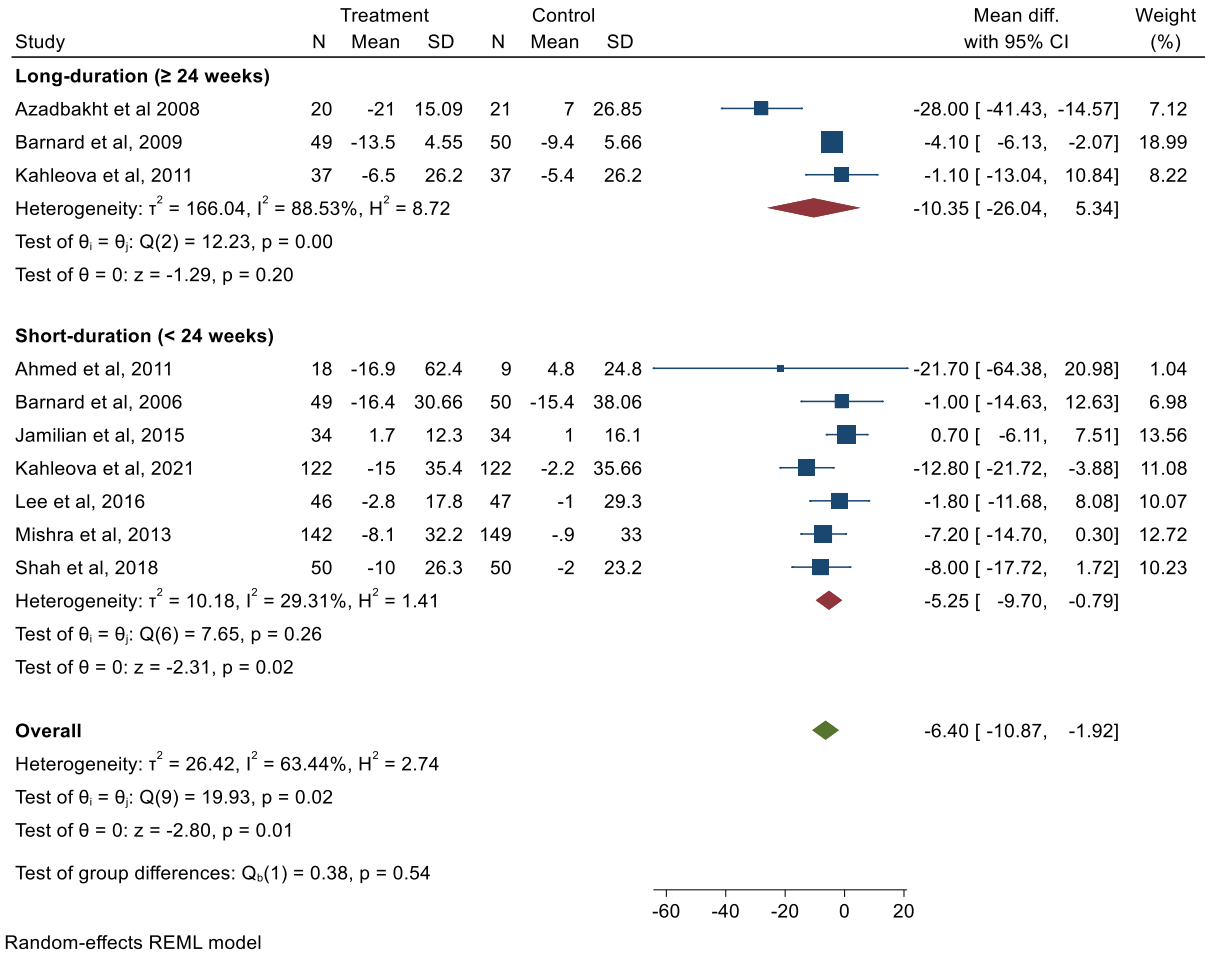
Appendix 3: Database search: Search string in **Cochrane Library**

Step	Query	Number of hits
1	("meat analogues"):ti,ab,kw OR (vegan):ti,ab,kw	253
2	("meat replac*"):ti,ab,kw OR ("non-animal protein"):ti,ab,kw OR ("meat alternatives"):ti,ab,kw OR ("protein alternatives"):ti,ab,kw OR (vegetarian):ti,ab,kw	660
3	("non-meat diet"):ti,ab,kw OR ("plant protein"):ti,ab,kw OR ("meat reduc*"):ti,ab,kw OR ("sustainable diet"):ti,ab,kw OR ("plant-based protein*"):ti,ab,kw (Word variations have been searched)	518
4	(#1 OR #2 OR #3)	1,288
5	("high meat intake"):ti,ab,kw OR ("normal diet"):ti,ab,kw OR ("animal foods"):ti,ab,kw OR (omnivor*):ti,ab,kw OR (meat):ti,ab,kw	3,281
6	("animal protein"):ti,ab,kw OR ("meat rich diet"):ti,ab,kw OR ("meat-based diet"):ti,ab,kw	271

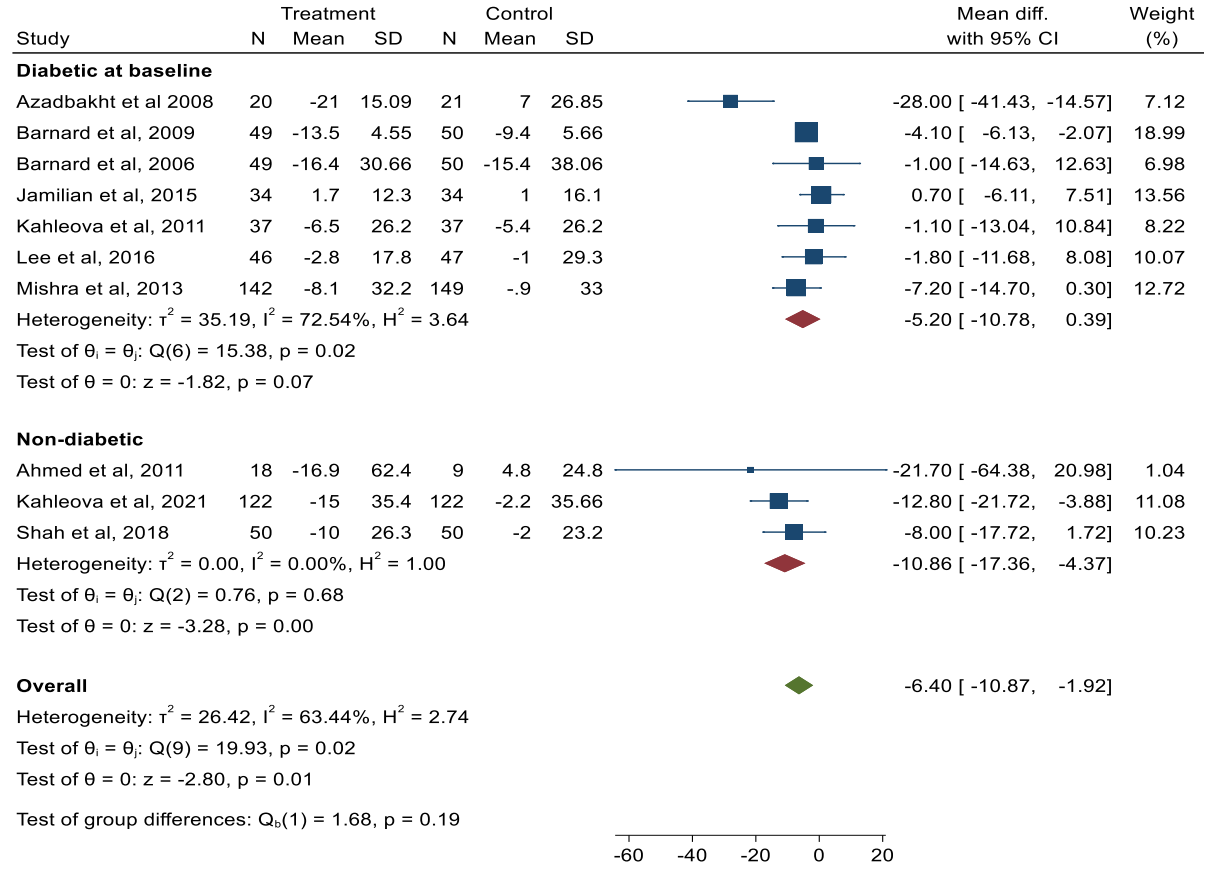
Step	Query	Number of hits
7	(#5 OR #6)	3,460
8	(glucose):ti,ab,kw OR (HbA1c):ti,ab,kw OR (Hb1c):ti,ab,kw OR (A1c):ti,ab,kw OR (glycohemoglobin):ti,ab,kw	78,450
9	("diabetes mellitus"):ti,ab,kw OR ("glycated hemoglobin"):ti,ab,kw OR ("serum glucose"):ti,ab,kw OR ("type 2 diabetes"):ti,ab,kw OR (T2DM):ti,ab,kw	76,940
10	("glucose-biomarker"):ti,ab,kw OR ("blood-sugar"):ti,ab,kw OR (diabet*):ti,ab,kw	103,964
11	(#8 OR #9 OR #10)	136,191
12	("lipid marker*"):ti,ab,kw OR ("high density lipoprotein"):ti,ab,kw OR (HDL):ti,ab,kw OR ("low density lipoprotein"):ti,ab,kw OR (LDL):ti,ab,kw	33,219
13	(hypercholesterolemia):ti,ab,kw OR ("LDL-cholesterol"):ti,ab,kw OR ("HDL-cholesterol"):ti,ab,kw OR ("lipid biomarker*"):ti,ab,kw	17,447

Step	Query	Number of hits
14	(#12 OR #13)	**36,344**
15	(#4 OR #7)	**4,386**
16	(#15 AND #11)	**890**
17	(#15 AND #14)	**537**
18	(#16 OR #17)	**1,215**
Total:		**1,215**

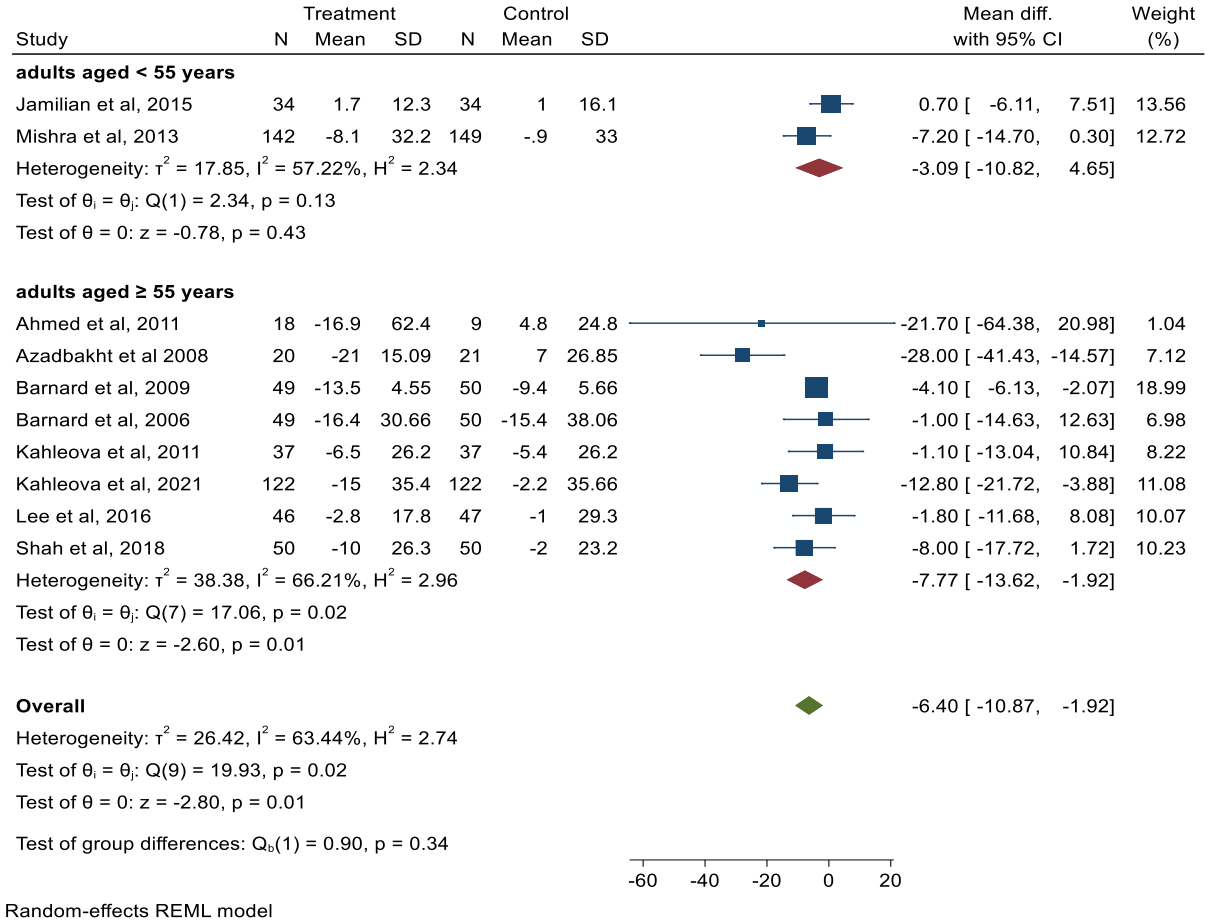
Appendix 4: Sub-group analysis on study duration; **LDL-c**. MD is presented as mg/dl



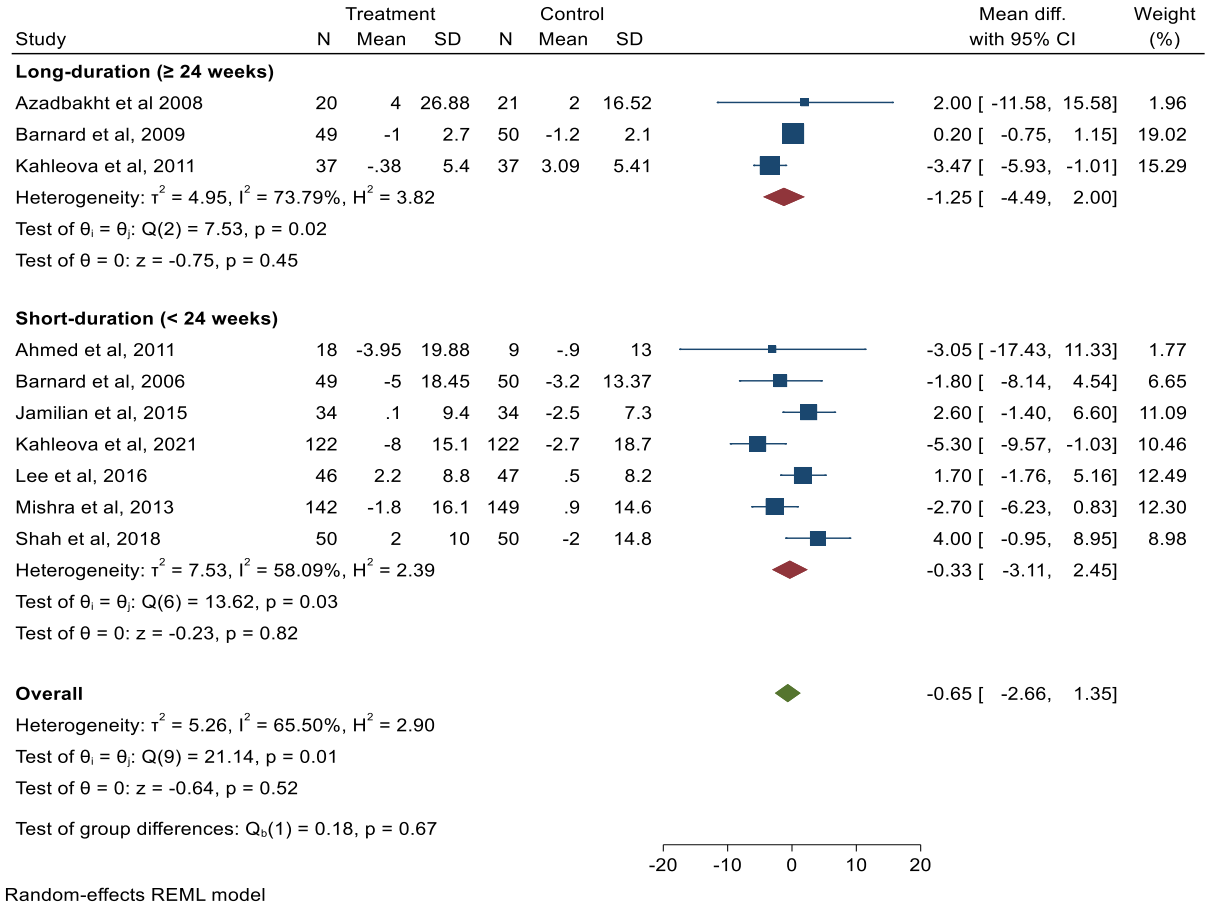
Appendix 5: Sub-group analysis on diabetes status; **LDL-c.** MD is presented as mg/dl



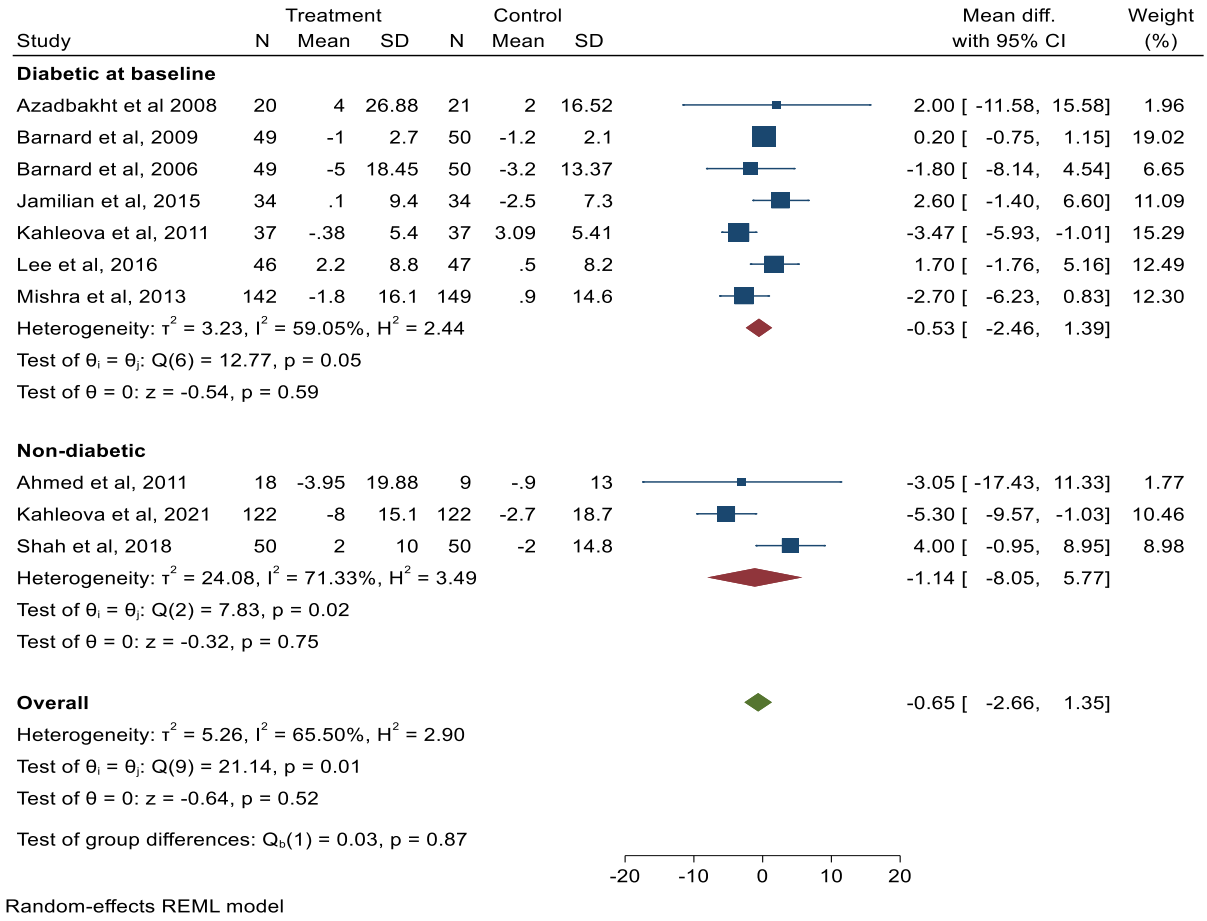
Appendix 6: Sub-group analysis on age-group; **LDL-c**. MD is presented as mg/dl



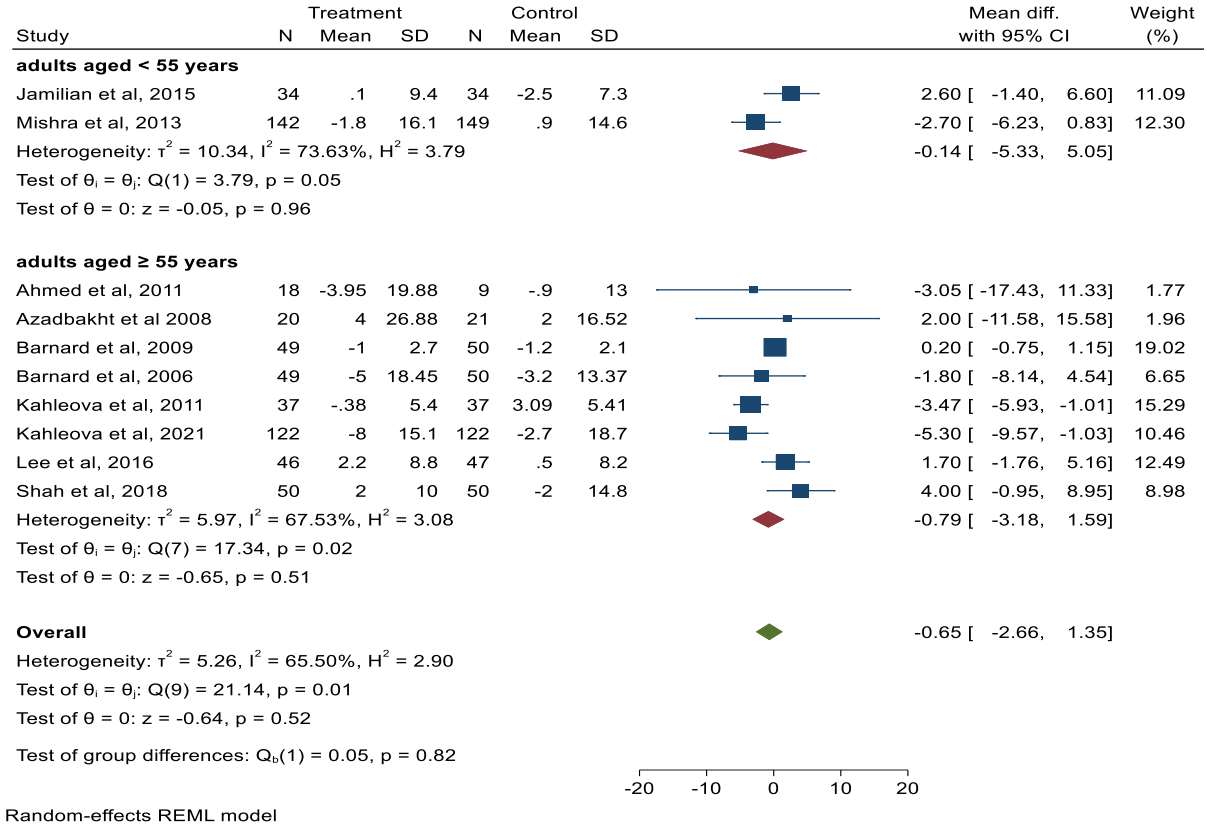
Appendix 7: Sub-group analysis on study duration; **HDL-c**. MD is presented as mg/dl



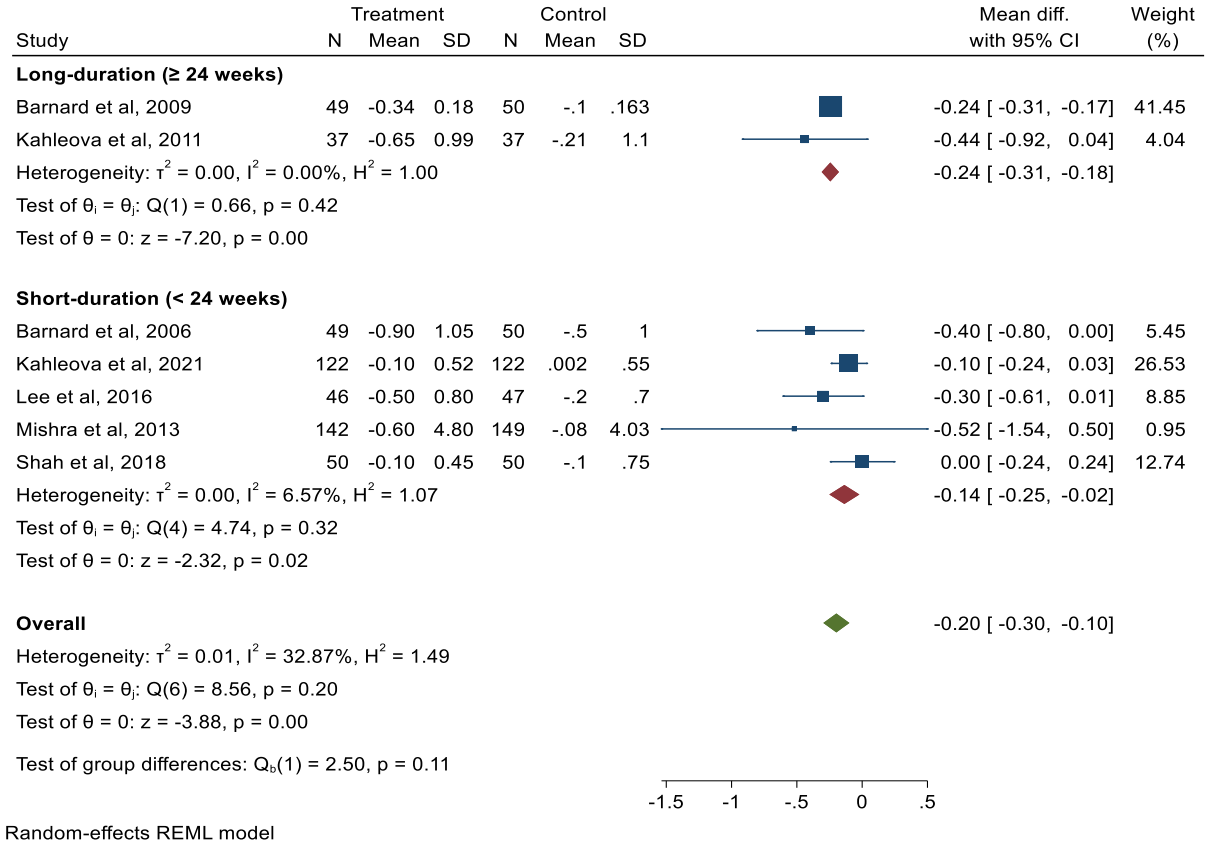
Appendix 8: Sub-group analysis on diabetes status; **HDL-c**. MD is presented as mg/dl



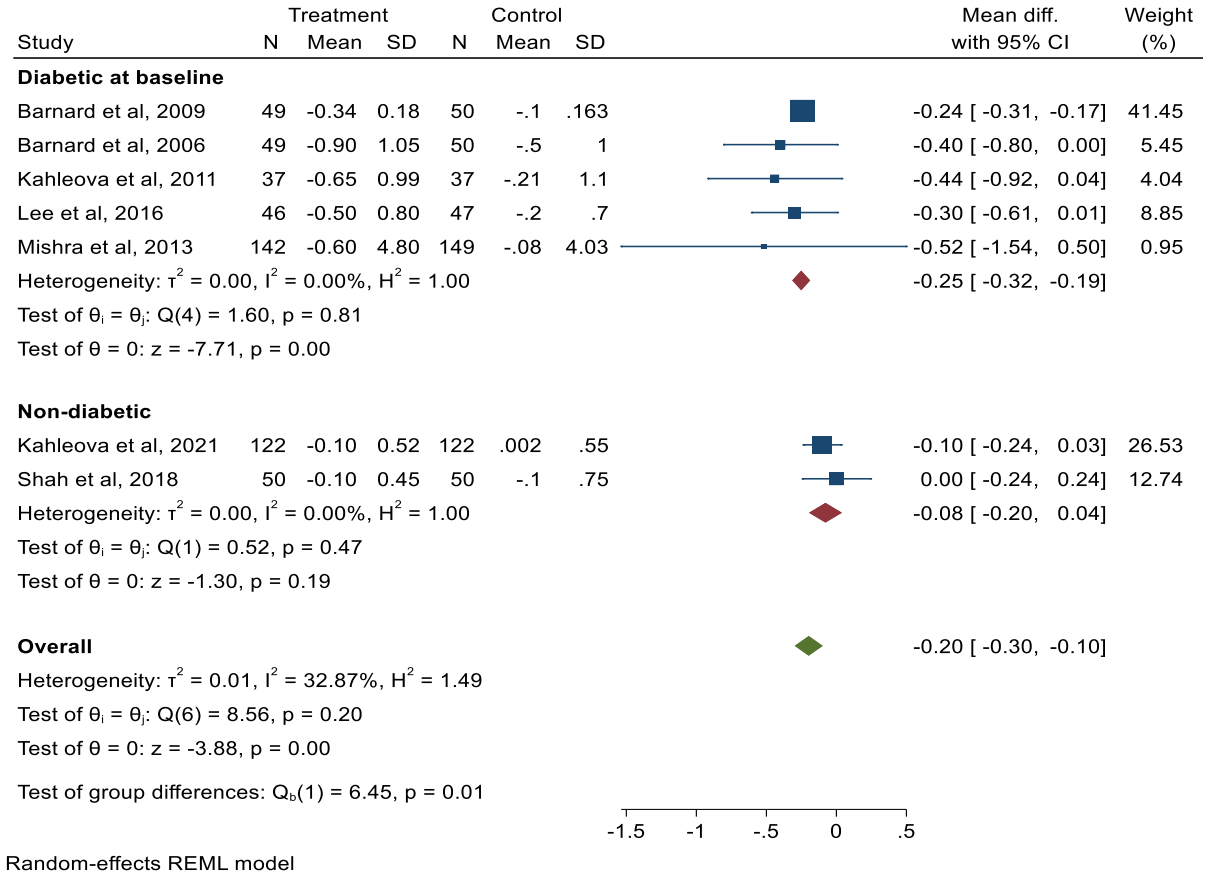
Appendix 9: Sub-group analysis on age-group; **HDL-c**. MD is presented as mg/dl



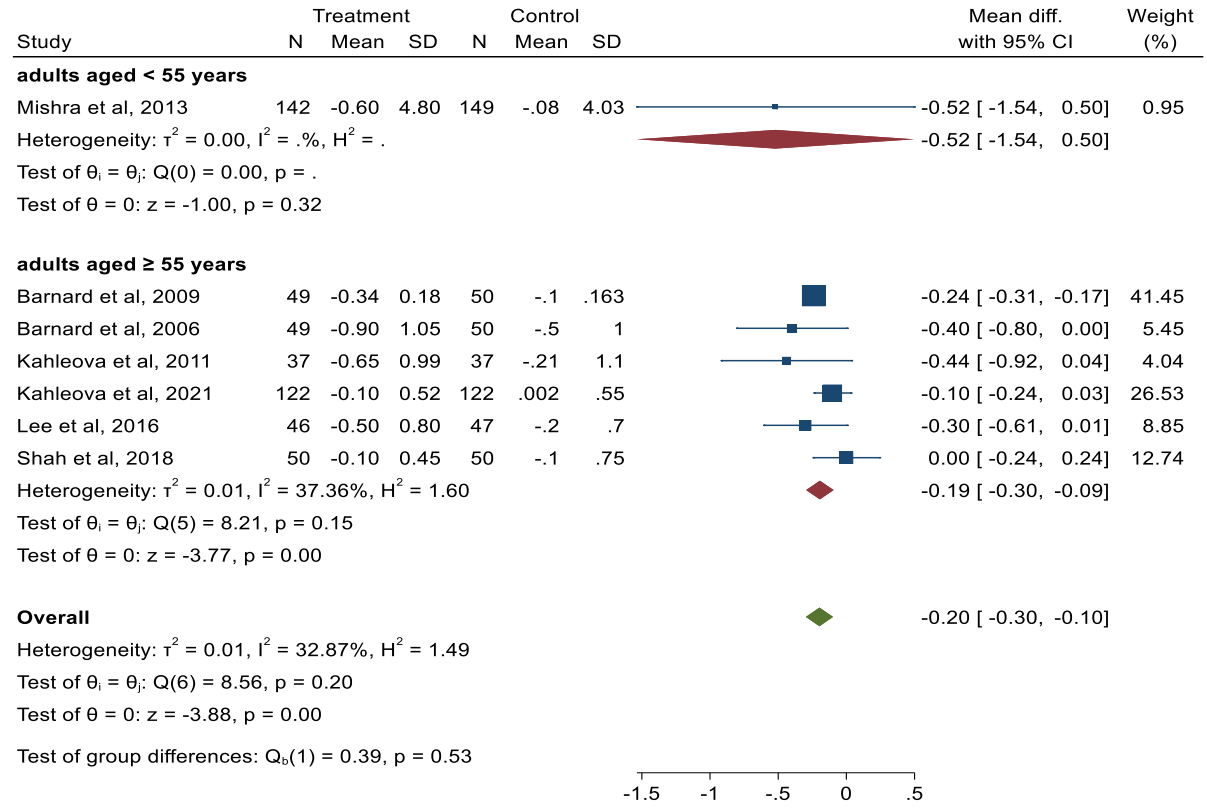
Appendix 10: Sub-group analysis on study duration; **HbA1c**. MD is presented as mg/dl



Appendix 11: Sub-group analysis on diabetes status; **HbA1c.** MD is presented as mg/dl



Appendix 12: Sub-group analysis on age-group; **HbA1c**. MD is presented as mg/dl



Appendix 13: Risk of bias on studies reporting data on **LDL-c** and **HDL-c**

Unique ID	Study ID	Experimental	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
Lee, Y. M. 2016	8	Meat-reduced diet	Meat-rich diet,	LDL and HDL	1	!	+	+	+	+	!
Ahmed et al, 20	1	Meat-reduced diet,	Meat-rich diet,	LDL and HDL	1	+	+	+	+	!	+
Azadbakht et al,	2	Meat-reduced diet,	Meat-rich diet,	LDL and HDL	1	+	+	+	+	+	+
Barnard et al, 2(	3	Meat-reduced diet,	Meat-rich diet,	LDL and HDL	1	!	+	+	+	+	+
Barnard et al, 2(	4	Meat-reduced diet,	Meat-rich diet,	LDL and HDL	1	!	+	+	+	+	+
Jamilian et al, 2(	5	Meat-reduced diet,	Meat-rich diet,	LDL and HDL	1	+	+	+	+	+	+
Kahleova et al, 2	6	Meat-reduced diet,	Meat-rich diet,	LDL and HDL	1	+	+	+	+	+	+
Kahleova et al, 2	7	Meat-reduced diet,	Meat-rich diet,	LDL and HDL	1	+	+	+	+	+	+
Mishra et al, 20	9	Meat-reduced diet,	Meat-rich diet,	LDL and HDL	1	+	+	+	+	!	!
Shah et al, 2018	10	Meat-reduced diet,	Meat-rich diet,	LDL and HDL	1	+	+	+	+	!	+

Appendix 14: Risk of bias on studies reporting data on **HbA1c**

Unique ID	Study ID	Experimental	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
Lee, Y. M. 2016	1	Meat-reduced diet	Meat-rich diet	HbA1c	1	!	+	+	+	+	!
Barnard et al, 20	2	Meat-reduced diet	Meat-rich diet	HbA1c	1	!	+	+	+	+	+
Barnard et al, 20	3	Meat-reduced diet	Meat-rich diet	HbA1c	1	!	+	+	+	+	+
Kahleova et al, 2	4	Meat-reduced diet	Meat-rich diet	HbA1c	1	+	+	+	+	+	+
Kahleova et al, 2	5	Meat-reduced diet	Meat-rich diet	HbA1c	1	+	+	+	+	+	+
Mishra et al, 201	6	Meat-reduced diet	Meat-rich diet	HbA1c	1	+	+	+	+	!	!
Shah et al, 2018	7	Meat-reduced diet	Meat-rich diet	HbA1c	1	+	+	+	+	!	+