Abating abdominal adiposity: Modifiable lifestyle risk factors for visceral and liver fat deposition

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ABSTRACT

The prevalence of obesity is increasing in most places in the world. In particular abdominal obesity is a well-established risk factor for cardiometabolic diseases. This excess risk due to abdominal obesity is due to fat in the visceral area and in and around the organs (ectopic fat), such as in the liver. The main aim of this thesis was therefore to study whether lifestyle changes can reduce the amount of visceral fat and liver fat.

Firstly, from a systematic review and meta-analysis of randomized controlled trials we concluded that a diet high in proteins decreases liver fat compared with a diet high in carbohydrates. A diet high in fat, however did not lead to changes in liver fat compared with a diet high in carbohydrates. Within fat types, saturated fat led to more liver fat accumulation than unsaturated fat. Secondly, we studied diet at multiple levels in the Netherlands Epidemiology of Obesity study, which is a population based cohort study of middle-aged men and women in the Leiden area with directly assessed measured of adiposity. When studying food groups rather than nutrients, we observed that consumption of sweet snacks was positively associated with liver fat content, also after we took total body fat into account. Likewise, consumption of fruit and vegetables and plant-based fats and oils was associated with a reduced amount of visceral fat. Adherence to the current Dutch dietary guidelines, as indicated by a high score on the 15-component Dutch Healthy Diet Index, was associated with less total body fat, less visceral fat and less liver fat. The associations with visceral fat and liver fat remained present after adjustment for total body fat, which suggests that the associations are indeed specific for visceral and liver fat rather than merely representing associations with overall adiposity. These associations were not driven by one component in particular, which stresses the importance of an overall healthy diet. When we studied alcohol intake separate from other dietary components, each additional serving of alcohol per day was also associated with an increase in liver fat. Furthermore , when one alcoholic serving is replaced with a non-alcoholic one, liver fat is also reduced . However, when alcoholic beverages were replaced with sugar sweetened beverages of the same caloric content, liver fat amounts were the same, whereas replacement with milk was associated with a reduced amount of liver fat. Lastly, we observed that objectively measured sedentary time was associated with an increase in total body fat, visceral fat and liver fat. Replacing 30 minutes of sedentary time per day with moderate to vigorous physical activity, but not light physical activity was associated with reduced total body fat, visceral fat and liver fat. These associations with visceral fat and liver fat disappeared after additional adjustment for total body fat, which indicates there is no extra effect on visceral fat and liver fat beyond effects via total body fat.

The results described in this thesis strongly hint towards the importance of considering diet as a whole, instead of focusing on separate components, which is in line with the current dietary guidelines. Sedentary behaviour should be replaced with moderate to vigorous physical activity rather than light physical activity. Alcohol should not be replaced with sugar sweetened beverages, but rather with milk, coffee or tea.

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General introduction, study population and outline of this thesis

The objective of this thesis was to study the role of dietary habits and physical activity in abdominal fat accumulation, which is a well-established risk factor for cardiometabolic diseases. This general introduction describes the epidemiology and historical perspective of overweight and obesity, the current knowledge about how diet and physical activity may influence visceral fat and liver fat, and how the research described in this thesis may contribute to addressing the gaps in knowledge that still exist.

Overweight and obesity: prevalence and relation to disease

Obesity is characterized as a condition in which excess energy is stored in the form of triglycerides in adipose tissue, which may ultimately cause health impairment [1]. In order to classify obesity the World Health Organization uses the body mass index (BMI). It is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2). Based on this index, the World Health Organization has made the following classification: underweight (<18.5 kg/m^2), normal weight (18.5-24.99 kg/m^2) and overweight (>25.0 kg/m^2). Because management options regarding prevention and treatment of obesity differ above a BMI of 35, the overweight category can be further subdivided into the following categories: preobese (25.0-29.99 kg/m^2), obese class I (30.0-34.99 kg/m^2), obese class II (35.0-39.99 kg/m^2) and obese class III (≥40.0 kg/m^2). In a large systematic analysis of health examination surveys and epidemiological studies it has been shown that between 1980 and 2008 the age-standardized mean global BMI has increased by 0.5 kg/m^2 in both men and women per decade [2].

In 1980, the global prevalence of overweight was 25% and that of obesity 6%. These numbers have increased to 34% for overweight and 12% for obesity in 2008 [3]. In absolute numbers this represents an increase from 572 million adults with overweight worldwide in 1980 to almost 1.5 billion in 2008, of whom 508 million with obesity [3]. This number has continued to rise even further to 670 million adult obese individuals in 2016, of whom 390 million were women and 280 million men. By 2020 2.2 billion adults will have overweight and 1.1 billion of them obesity when recent secular trends are taken into account [4]. This trend is also visible in the Netherlands: in 2018 half of all adults (50.2%) had overweight and 15% of them had obesity [5].

Excess body fat is an established strong risk factor for multiple chronic diseases, such as type 2 diabetes, cardiovascular disease and certain types of cancer. In 2015, overweight and obesity accounted for 4 million deaths, which contributed to a little over 7% of the deaths from any cause that year. Overweight was also responsible for 120 million disability-adjusted life-years [6].

Obesity and weight gain have been associated with an increased risk of diabetes [7, 8], and those with a BMI of 40 or higher are 7 times more likely to be diagnosed with diabetes than those with a normal BMI [9]. The relation is so strong, that it is thought that 90% of type 2 diabetes is attributable to excess weight [10]. Both overweight and obesity are also related to the risk of developing cardiovascular risk factors such as hypertension, and as a consequence a BMI of 25 accounts for 35% of hypertension diagnoses in men and 60% in women [11]. An increased risk due to overweight was also found for coronary heart disease and cerebrovascular disease in both men and women in large cohort studies such as the Nurses' Health Study, the Dallas Heart Study and the Physicians' Healthy Study [12]. At a global level, 41% of high BMI-related deaths were caused by cardiovascular disease among people with obesity, and diabetes was the second largest cause [6]. In both men and women, a high BMI has also been associated with an increased risk of death due cancer of the esophagus, colon and rectum, liver, gallbladder, pancreas and kidney, and with non-Hogdkin's lymphoma and multiple myeloma [13]. A dose-response relationship, in which the risk of death increases with higher BMI, was found for stomach and prostate cancer in men and breast, uterus, cervix and ovary cancer in women [13].

Multiple studies have found that a high BMI is a strong risk factor for end stage renal disease [14]. Obesity has also been linked to knee osteoarthritis due to the increased load on these weight-bearing joints, and other joint-related disorders such as gout [12]. Additionally, excess body weight increases the risk of pancreatitis, ischemic dementia, Alzheimer's disease and psychological problems, such as mood and anxiety disorders [12].

Body fat distribution and ectopic fat

Although BMI is a widely-used and easy to measure proxy for overall adiposity, it does not take body composition into account. A high BMI does not necessarily indicate a high body fat percentage, as it might also be the consequence of larger muscle mass. Moreover, BMI does not consider where exactly the fat is stored in the body.

Already in 1947, Vague discovered that patients with hypertension, diabetes or cardiovascular disease were not per definition more obese than those without complications [15]. He did, however, observe that individuals who had more fat stored in the trunk area had an increased risk of diabetes and cardiovascular disease. As a result, he identified two body shapes and formulated the terms *gynoid obesity* and *android obesity* [15]. Nowadays, we still use these terms: android obesity is characterized by a large waist circumference with the majority of the adipose tissue centering around the abdomen, more commonly found in men. In contrast, in a gynoid or pear shaped body type most adipose tissue is stored subcutaneously at the hips, buttocks and thighs, often going

hand in hand with a smaller waist circumference. This body type is more common in women.

In the early 1980s, another breakthrough in the field of obesity was accomplished. Björntorp and his group discovered that body shape, and thereby the regional accumulation of body fat, and the morphology of this fat were closely related to metabolic diseases [16]. Subsequently, they described that men with a high proportion of abdominal fat had a substantially increased risk of developing diabetes [17]. These discoveries have sparked the interest of the medical community and laid the foundation for the research on body fat distribution we do today.

After the development of imaging techniques such as computed tomography (CT), it became possible to scan the whole body and discriminate between different types of tissue. Researchers from the University of Osaka were the first to use the CT for this purpose, and were able to distinguish the fat located in the abdominal cavity, or visceral fat, from the fat located subcutaneously [18]. A few years later, in 1987, they were the first to show the detrimental effects of viscerally stored adipose tissue, by demonstrating that subjects with more visceral adipose tissue displayed higher fasting plasma triglycerides levels and higher plasma glucose responses following an oral glucose challenge than those with the same BMI but fat mainly stored subcutaneously [19]. In 1989, Seidell showed that visceral fat area as measured with CT was also associated with serum triglycerides, plasma insulin, glucose and diastolic and systolic blood pressure [20].

These landmark studies in combination with more recent developments have shown that the majority of lipids accumulate in subcutaneous adipose tissue, which is located just below the skin and amounts to 82-97% of total body fat. Subcutaneous adipose tissue has the capability to expand when there is a positive energy balance. However, the response to excess caloric intake might vary, and there is considerable variation between individuals where the fat is stored [21]. Lipids can also accumulate in visceral adipose tissue, which is located deeper in the body and situated around the organs, and amounts to 10 to 15% of all fat [22]. Additionally, lipids can be stored in non-adipose tissue cells, such as in the liver (intrahepatic fat) or the muscles (intramuscular fat). This is referred to as ectopic fat.

There are large sex-related differences in the location of fat deposition. On average, women have a higher percentage of body fat than men for the same BMI and are more likely to store fat subcutaneously in the femoral gluteal region. Men, however, store more fat in the visceral area [23, 24]. These differences are caused by multiple factors, such as differences between men and women in basal fatty acid oxidation, postprandrial fatty acid storage, and regional differences in the regulation of lipolysis [23]. As postmenopausal women are more likely to store fat viscerally than premenopausal women, testosterone levels also seem to be important in body fat distribution [25].

Furthermore, studies in identical twins have shown that the susceptibility to store fat either viscerally or subcutaneously is partly determined by genetics [26-28], and multiple loci associated with visceral adipose tissue have been identified in genome wide association studies, of which some appear sex-specific [29]. Additionally, age, ethnicity, physical activity and levels of glucocorticoids have also been associated with visceral fat accumulation [30]. Due to the large individual variation in adipose tissue depots and various factors contributing to these differences in deposition, obesity is a surprisingly heterogeneous condition iwhenit is defined based on BMI alone [30], as it has been shown that patients with similar BMI values present different levels of health risk [31].

In parallel to the increase in obesity over the past decades, the prevalence of non-alcoholic fatty liver disease (NAFL) also continues to rise and is now present in 25% of the general adult population, and 65% to 85% in adults with obesity [32]. Non-alcoholic fatty liver disease is defined as having more than 5.56% of liver fat not due to excessive alcohol consumption [33] and may lead to non-alcoholic steatohepatitis (NASH) and liver cirrhosis. It also increases the risk of end-stage liver disease and liver-related and all-cause mortality [34-37]. Currently, it is the leading cause of chronic liver diseases worldwide [38], and is also strongly related with the metabolic syndrome [39] and cardiovascular diseases [40]. In previous research, visceral adipose tissue and hepatic triglyceride content have both been associated with insulin resistance, metabolic risk factors and cardiovascular disease [41-44]. Since the first state of NAFL, simple steatosis, is still reversible, adequate treatment is needed [45, 46].

The overflow hypothesis explains fat accumulation in liver and visceral area, and states that the body's response to excess calories may determine the risk of the metabolic syndrome [47]. When the caloric surplus is led into the subcutaneous adipose tissue, which is sensitive to insulin, the individual is unlikely to develop the metabolic syndrome as the subcutaneous fat can expand. When, however, this adipose tissue is not functioning properly or if it is insulin resistant with an insufficient capacity to store the extra energy, the fatty acids will be stored in visceral adipose depots, in turn leading to ectopic fat disposition. Therefore, according to the lipid overflow hypothesis, accumulation of visceral fat is a marker of dysfunctional subcutaneous fat [30]. In the liver, the increased free fatty acid flux observed in individuals with obesity [47] leads to

increased hepatic lipase activity. In turn, this causes reduced hepatic degradation of insulin and apolipoprotein B and increased hepatic glucose production, leading to glucose intolerance [48]. Moreover, visceral fat is also associated with an increased risk of respiratory diseases such as sleep apnoea or chronic obstructive pulmonary disease (COPD), dementia, reduced bone density, polycystic ovary syndrome and different types of cancer [49]. It decreases adiponectin secretion, and together with liver fat thereby brings about hypertension, insulin resistance, dyslipidaemia, and ultimately atherosclerosis [50]. Both visceral fat and liver fat have been associated also with coronary artery disease and cardiovascular disease [41, 43].

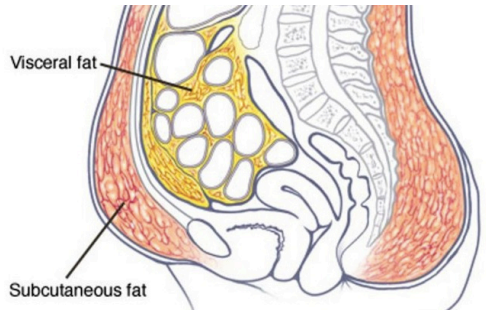

Figure 1. Subcutaneous fat and visceral fat in the abdomen

Diet as a modifiable risk factor for visceral fat and liver fat

Visceral fat and liver fat are key targets in the prevention and treatment of cardiometabolic diseases. Due to a lack of drug-based treatments, modifiable lifestyle factors such as dietary habits and physical activity are key when it comes to the prevention and treatment of abdominal obesity [45]. Dietary habits can be studied on multiple levels. To understand how particular components of food are related to disease, studying diet on a micro- or macronutrient level is useful. The main focus regarding decreasing body weight concerns caloric restriction. However, besides diet quantity, diet quality may also be important for health. An overfeeding trial of saturated and polyunsaturated fatty acids has shown distinct effects on visceral fat and liver fat [51], indicating the importance of dietary macronutrient composition for the accumulation of adipose tissue. Moreover, it has been proposed that specific nutrients, such as fructose, increase hepatic de novo lipogenesis [52]. Increased lipolysis of visceral fat can contribute to an increased flux of free fatty acids in the liver, which may ultimately lead to accumulation of triglycerides and other lipids in the liver [53]. Although some meta-analyses have been performed on specific micronutrients in relation to NAFL, results were inconclusive [54]. For

subcutaneous and visceral fat, one systematic review on the effects of dietary aspects has already been published [55], and described an inverse relation between intake of medium chain triglycerides and dietary patterns recognized as healthy and subcutaneous fat and visceral fat. Dietary fiber and calcium were found to be negatively associated with visceral fat only. For liver fat, however, such an overview does not yet exist. Besides omega-3 polyunsaturated fatty acids and fructose consumption, little is known about the association between other macronutrient and macronutrient subtypes and liver fat.

As foods are not merely the sum of their nutrients, health effects of diet can be studied at the level of foods and food groups rather than single nutrients [56-58]. The food matrix may play a role, or interactions between the separate nutrients within a food item might occur [57-59]. In line with this, several European countries and the United States have published dietary guidelines that are based on food products and groups instead of single nutrients [60]. Previous studies have shown that major food groups such as meat, dairy and fruit and vegetables are associated with body weight [61], diabetes [62] and cardiometabolic diseases such as coronary heart disease (CHD) [63]. However, it remains unclear to what extent these food groups are specifically associated with visceral fat and liver fat. Knowledge on these potential associations might contribute to the development of new preventive guidelines regarding healthy dietary habits, or the adjustment of current guidelines in relation to cardio metabolic diseases.

Combining multiple food items or groups makes it possible to study dietary patterns as a whole. Dietary guidelines that have been developed on the basis of previous research state the optimal consumption of several food items and food groups. Adherence to these guidelines can be assessed using an index. Numerous dietary indices of adherence to a healthy diet have been developed recently, among which the (Alternative) Healthy Eating Index (HEI) [64], the Healthy Diet Indicator [65] and the Diet Quality Index (DQI) [66]. Research has shown that a higher index, indicating a better adherence to the dietary guidelines and therefore a healthier diet, is associated with a lower risk of obesity, cardiovascular disease and all-cause mortality [67]. In the Netherlands, the Health Council of the Netherlands has developed the Dutch Guidelines for a Healthy Diet, of which the newest version appeared in 2015 [68]. These new guidelines are mostly foods-based and describe the optimal consumption for food groups such as fruit and vegetables and dairy, but also consumption of unsalted nuts, green tea and filtered coffee. Adherence to these guidelines can be assessed using the Dutch Healthy Diet Index, which scores each component of the Dutch Guidelines for Healthy Diet. Using this index makes it possible to study to what extent adherence to the guidelines is associated with multiple health-related outcomes. Although the 2015 DHD-index is still recent, a higher score on

the Dutch Healthy Diet Index 2015 has been associated with a decreased risk of stroke, chronic obstructive pulmonary disease, colorectal cancer and all-cause mortality [69]. However, it remains unclear to what extent adherence to the 2015 Dutch Guidelines for a Healthy Diet is specifically associated with the amount of visceral fat and liver fat.

Alcoholic and non-alcoholic beverages in relation to liver fat

Excessive alcohol consumption is a well-established risk factor for both hepatic steatosis (liver fattening) and liver disease [70]. Although current guidelines aim at preventing or reducing liver fat accumulation recommend to refrain from heavy alcohol consumption, it remains unclear whether moderate alcohol consumption should also be discouraged. Several studies have shown a beneficial effect of light to moderate alcohol consumption in relation to fatty liver and extra-hepatic complications [71-74]. However, a study using a genetic variant in the alcohol dehydrogenase gene as a proxy of long-term alcohol exposure showed no beneficial effect of moderate alcohol consumption on the severity of non-alcoholic fatty liver disease [75]. Even when alcohol use is discouraged, it it is unclear with what beverages patients with non-alcoholic fatty liver should replace their alcoholic drinks. We hypothesize that non-alcoholic energy containing beverages may also contribute to liver fat accumulation, as calories from these beverages contribute to the total energy intake and liquid food leads to less satiety and more postprandial hunger [76]. Knowledge on the association between different beverages and their mutual replacement with liver fat may contribute to lifestyle guidelines for both primary and secondary prevention.

Physical activity in relation to abdominal fat

Besides healthy dietary habits, physical activity is a key modifiable risk factor for obesity and cardiovascular disease. Energy balance within the body reflects a harmony between of energy intake, energy expenditure and energy storage. While diet provides the energy intake, energy expenditure consists of three components: the energy it takes to fuel the body at rest (resting metabolic rate, approximately 60-75% of total energy expenditure), the energy it costs to absorb and metabolize the food that is consumed (thermic effect of food, approximately 10% of total energy expenditure) and the energy expended by undertaking physical activity (approximately 15-30% of total energy expenditure) [77, 78]. When the energy intake is greater than the energy expenditure over a longer period of time, this will lead to excess storage of fat and thereby an increase in body weight [79]. Physical activity has been defined as 'any bodily movement produced by skeletal muscles that results in energy expenditure [80] and can be subdivided into multiple activity intensities. activity levels are usually expressed in Metabolic Equivalents of Task (METs), which assign an intensity value to each specific activity [81]. Light physical activity is defined as activities lower than 3.0 METs and includes activities such as sitting (1.5 MET), walking slowly (2.0 MET) or playing a musical instrument (2.5 MET). Moderate physical activity is defined as 3.0 to 6.0 MET, such as cleaning (3.0 MET), playing recreational badminton (4.5 MET) or mowing the lawn (5.5 MET). All activities above 6.0 MET are defined as vigorous [82].

Most European guidelines on physical activity recommend to perform at least 150 minutes of moderate to vigorous physical activity per week. Additionally, they state sedentary time should be limited [83], as this behaviour has been associated with increased risk of type 2 diabetes, cardiovascular disease and cancer [84]. A meta-analysis has shown that exercise is able to reduce both visceral fat and liver fat, although the studies that were included focused on structured exercise instead of habitual daily activities. Therefore, the evidence on the potential association between these so-called unstructured activities and sedentary time as assessed with accelerometry and different adipose depots is still lacking. Furthermore, less time spent sedentary inevitably means more time is spent performing other activities. In the study of a decrease of time spent in particular activity, it is therefore important to take into account with which activity this is being replaced (e.g., replacing sedentary time with the same time spent on moderate to vigorous physical activity). This can be done using isotemporal substitution analysis. However, most studies using this type of statistical model use surrogate outcomes for adiposity such as body mass index or waist circumference instead of directly assessed measures of adiposity [85]. Only one study has combined objectively measured physical activity and direct measures of visceral fat, and observed that isotemporal substitution of one hour per day of sedentary and light intensity physical activity with moderate to vigorous physical activity was associated with reduced visceral fat [86]. However, liver fat was not assessed in this study and no adjustment for total body fat was performed. Therefore, evidence on how replacing sedentary time with other activities is associated with directly assessed visceral fat and liver fat is still largely lacking.

OUTLINE OF THIS THESIS

Although many previous studies have investigated the health-related consequences of a healthy lifestyle, there is still much to be learned. Up to date, not much is known about how diet and physical activity affect visceral fat and liver fat. Therefore, the objective of this thesis was to study the role of dietary habits and physical activity in abdominal fat accumulation, more specifically in the visceral area and in the liver.

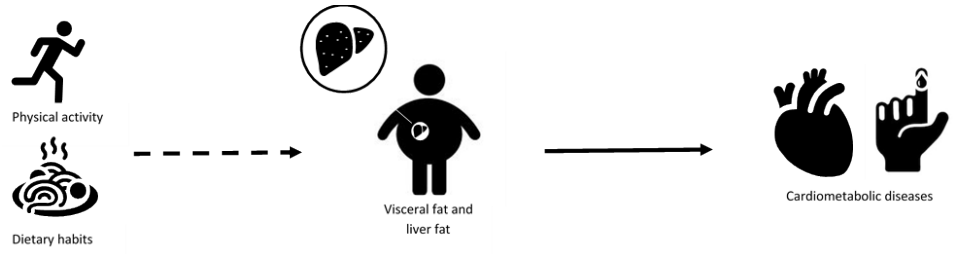

Figure 2. Visual representation of hypothesized association between dietary habits and physical activity and visceral fat and liver fat, ultimately leading to cardiometabolic diseases

In order to prevent non-alcoholic fatty liver from progressing to more severe forms of hepatic fat storage, such as non-alcoholic steatohepatitis or even liver cirrhosis, adequate treatment is needed.

The current treatment for non-alcoholic fatty liver disease is mainly focused on weight loss by means of calorie restricted diets [87-89]. However, besides diet quantity in the form of caloric restriction, the macronutrient composition of a diet may be important. In **Chapter 2** we performed a systematic review and meta-analysis on the effect of dietary macronutrient composition on liver fat in randomized controlled trials. Studies included in this meta-analysis compared diets high in one macronutrient and low in another with diets with the opposite composition and their effect on liver fat content.

As foods are not merely the sum of their nutrients, studying foods and food groups may be important in relation to multiple health outcomes [57] and is becoming more important in the development of dietary guidelines. In **Chapter 3** we examined the association between dietary intake of certain food groups, such as meat, fruit and vegetables and dairy, and visceral fat and liver fat specifically. We also investigated a finer categorization of these main food groups, to assess whether associations were driven by one component of that food group in particular.

Adherence to the Dutch Guidelines for a Healthy Diet, which are dietary guidelines that aim to decrease the risk of chronic diseases [90], can be measured using the Dutch Healthy Diet Index [91]. In **Chapter 4**, we aimed to examine whether the 2015 Dutch Healthy Diet Index is associated with both total body fat, visceral fat and liver fat. By leaving each component out one at a time, we tried to examine which component is most important in these associations.

Besides consumption of certain nutrients and food items or groups, excessive alcohol consumption is a well-known risk factor for liver fattening and liver disease [70]. Current guidelines to prevent or reduce liver fat accumulation recommend that heavy drinking should be refrained from [92]. However, it remains unclear what beverages should replace the alcohol when people are advised to refrain from further alcohol consumption. We hypothesized that energy-containing non-alcoholic beverages, such as sugar sweetened beverages, also contribute to the accumulation of liver fat, and investigated the association between both alcoholic and non-alcoholic beverages and liver fat in **Chapter 5**. We also assessed how replacing alcohol with non-alcoholic beverages is associated with liver fat.

Besides dietary habits, physical activity is another key modifiable risk factor for obesity and cardio metabolic diseases. In **Chapter 6** we investigated different levels of physical activity, such as sedentary behaviour, light, moderate and vigorous physical activity, in relation to total body fat, visceral fat and liver fat. In this study we have also used isotemporal substitution analysis to study the association between replacing 30 minutes of sedentary time with 30 minutes of another activity and the body fat measurements.
Lastly, **Chapter 7** summarizes important findings of this thesis and discusses their interpretation and clinical implications.

Population and design of the Netherlands Epidemiology of Obesity study

All studies described in this thesis, except for the meta-analysis, have been performed in the Netherlands Epidemiology of Obesity (NEO) study. This is a population-based prospective cohort study in 6 671 individuals aged 45 to 65 years, with an oversampling of persons with a BMI of 27 kg/m^2 or higher. All studies described in this thesis are based on the baseline measurements and therefore of a cross-sectional nature. Men and women aged between 45 and 65 years with a self-reported BMI of 27 kg/m^2 or higher living in the greater area of Leiden (in the West of The Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI, allowing for a reference distribution of BMI. To be able to make inferences on the general population, we weighted all analyses towards the BMI distribution of the Dutch general population using the distribution of the Leiderdorp participants. Consequently, all results described in this thesis apply to the general population.

Participants visited the NEO study center of the Leiden University Medical Center after an overnight fast. Prior to the NEO study visit, participants completed a questionnaire about demographic, lifestyle, and clinical information, in addition to a food frequency questionnaire. At the study center, the participants completed a screening form, asking

about anything that might create a health risk or interfere with magnetic resonance imaging (MRI) (most notably metallic devices, claustrophobia, or a body circumference of more than 1.70 m). Of the participants who were eligible for MRI, approximately 35% were randomly selected to undergo direct assessment of abdominal fat. In total, 2,580 participants had a valid measurement of visceral adipose tissue by MRI, and 2,083 participants of hepatic triglyceride content by MRS. These imaging modalities allow direct assessment of visceral adipose tissue and hepatic triglyceride content, which is a unique feature of the NEO study as most epidemiological studies use composite measures to estimate abdominal adiposity. An overview of the main methods to assess overall body fat and abdominal fat can be found in **Table 1**.

The Medical Ethics Committee of the Leiden University Medical Center approved the design of the study. All participants gave their written informed consent.

Table 1. Commonly used measures of overall body fat and abdominal fat

Measure	Instrument	Validity	Feasibility in large epidemiological studies	Advantages	Disadvantages
Overall body fat					
Weight (kg)	Questionnaire or scale	Low	High	Easily measured in large epidemiological cohorts	Does not discriminate body fat and fat free mass
BMI (kg/m²)	Questionnaire or scale	Low	High	Easily measured in large epidemiological cohorts	Does not discriminate body fat and fat free mass
Total body fat (%)	Bio impedance balance	High	Low	Provides indication in the amount of adipose tissue in body	Does not discriminate between subcutaneous and visceral fat
Abdominal fat					
Waist circumference (cm)	Measuring tape	Intermediate	High	Easily measured in large epidemiological cohorts	No direct measure of abdominal fat
Waist-hip-ratio	Measuring tape	Intermediate	High	Easily measured in large epidemiological cohorts	No direct measure of abdominal fat
aSAT (cm²)	Magnetic resonance imaging	High	Low	Direct measure of abdominal subcutaneous adipose tissue	Expensive and time-consuming
VAT (cm²)	Magnetic resonance imaging	High	Low	Direct measure of visceral adipose tissue	Expensive and time-consuming
HTGC (%)	Proton magnetic resonance spectroscopy	High	Low	Direct measure of hepatic fat	Expensive and time-consuming

aSAT, abdominal subcutaneous adipose tissue; BMI, body mass index; HTGC, hepatic triglyceride content; VAT, visceral adipose tissue.

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2

Dietary effects of macronutrients and macronutrient types on liver fat content in adults: a systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

Dietary macronutrient composition may affect hepatic liver content and its associated diseases, but the results from human intervention trials have been equivocal or underpowered. We aimed to assess the effects of dietary macronutrient composition on liver fat content by conducting a systematic review and meta-analysis of randomized controlled trials in adults. Four databases (PubMed, Embase, Web of Science and COCHRANE Library) were systematically searched for trials with isocaloric diets evaluating the effect of dietary macronutrient composition (energy percentages of fat, carbohydrates and protein, and their specific types) on liver fat content as assessed by magnetic resonance techniques, computed tomography or liver biopsy. Data on change in liver fat content were pooled by random or fixed-effects meta-analyses and expressed as standardized mean difference (SMD). We included 21 randomized controlled trials providing data for 25 comparisons on dietary macronutrient composition. A high-carbohydrate low-fat diet did not change liver fat content as compared with a low-carbohydrate high-fat diet (12 comparisons, SMD 0.01 (95% CI -0.36; 0.37)). Heterogeneity was substantial (I^2 67.8%, p<0.001). Unsaturated fat as compared with saturated fat reduced liver fat content (3 comparisons, SMD -0.75 (95% CI -1.11; -0.39)). A high-protein low-carbohydrate diet reduced liver fat content as compared with a low-protein high-carbohydrate diet (3 comparisons, SMD -0.32 (95% CI -0.58; -0.05)). Our meta-analyses showed that replacing carbohydrates with total fat on liver fat content was not effective, while replacing carbohydrates with proteins was. We showed that unsaturated fat consumption leads to less liver fat content compared with saturated fat consumption. Too few studies were included to perform separate meta-analyses on types of carbohydrates and proteins, and therefore more well-performed and well-described studies on the effect of types of carbohydrates and proteins on liver fat content are needed, especially studies comparing proteins with fats.

INTRODUCTION

Non-alcoholic fatty liver (NAFL) is clinically defined as a liver fat content of more than 5.6%, not due to excessive alcohol consumption [1]. It is a major cause of chronic liver disease worldwide, associated with an increased risk of liver- and cardiovascular disease-related mortality [2-5]. Moreover, obesity and other features of the metabolic syndrome such as dyslipidaemia, insulin resistance and diabetes mellitus, are associated with NAFL [6-10]. The prevalence of NAFL continues to rise [2, 3] and has been estimated at 25% in adults [2], and between 65% and 85% in adults with obesity [11].

Since NAFL is still reversible, adequate treatment is needed to prevent the development into more severe forms of hepatic fat storage such as non-alcoholic steatohepatitis (NASH)[12, 13]. Drug-based treatments are primarily recommended for patients with a later stage of NAFL, whereas lifestyle changes are a cornerstone in guidelines on treatment of NAFL, including weight loss, eating healthier, and increasing physical exercise [12]. To date, interventions on NAFL mainly focus on decreasing total body fat by recommending calorie restricted diets in overweight or obese patients [14-16]. However, besides diet quantity in the form of caloric restriction, macronutrient composition may be of importance, although evidence on this is scarce. Recent meta-analyses have shown that supplementation of omega-3 polyunsaturated fatty acids (PUFAs) is an effective intervention for reducing NAFL [17, 18].

Besides specific types of macronutrient such as omega-3 polyunsaturated fatty acids and fructose consumption, there are no meta-analyses on other macronutrients and other macronutrient types. In only one review on the effects of macronutrients on liver fat it has been described that a relatively high consumption of saturated fat increases the percentage of liver fat, whereas an increased consumption of refined sugars had no influence on liver fat [19]. However, the search of this review was limited and was not substantiated by a meta-analysis. Therefore, it remains unclear whether dietary macronutrients and their composition affect liver fat content. We aimed to assess the effect of dietary macronutrient composition on liver fat content, as measured by magnetic resonance imaging, proton magnetic resonance spectroscopy, computed tomography or liver biopsy, by performing a systematic review and meta-analysis of isocaloric randomized controlled trials in adults.

METHODS

This systematic review and meta-analysis on dietary macronutrient composition and liver fat content was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-) guidelines and the recommendations of the Cochrane Collaboration [20, 21]. The protocol is registered at PROSPERO with registry ID number 100356.

Eligibility criteria

Databases were systematically searched for eligible publications based on a priori determined eligibility criteria. We systematically searched for randomized controlled dietary intervention trials evaluating the effect of macronutrient composition on liver fat content in adults. Studies including healthy adults as well as patients with obesity, metabolic syndrome, (pre)diabetes, NAFL or NASH and/or cardiovascular disease, were considered eligible. Trials that included individuals with malignant diseases or with alcoholic, drug-induced, viral or genetic causes of liver injury, were excluded.

Both macronutrient comparisons (carbohydrates versus fat, carbohydrates versus protein, protein versus fat) and macronutrient types comparisons (types of fat, types of carbohydrates and types of protein) were assessed. Since several reviews and meta-analyses on omega-3 fatty acids and fructose have been published recently [17, 22-26], studies were excluded when the dietary intervention was primarily focused on these types of macronutrient comparisons. Studies that used hyper- or hypo-caloric interventions were only eligible when caloric intake was equal in both study arms. Furthermore, the interventions had to be provided for at least one week, since seven days of dietary intervention was deemed necessary to influence fat oxidation in the liver [27]. In addition, trials that involved co-interventions, such as exercise or other lifestyle interventions, were only included when similar in both arms of the trial. Trials solely providing their participants with dietary advice rather than food items, as well as trials presenting insufficient information on macronutrient composition were not eligible. Assessment methods of liver fat content were predefined: only trials in which liver fat content was measured by magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), computed tomography (CT) or liver biopsy were considered [28, 29].

Search strategy

We conducted a systematic search to identify eligible publications. In cooperation with a trained librarian (JWS), a detailed search strategy was composed for the four bibliographic databases: PubMed, Embase (OVID-version), Web of Science, and COCHRANE Library. The search query consisted of a combination of the following concepts: macronutrients (exposure terms), liver fat (outcome terms) and (randomized controlled) trials. The search strategy was adjusted for all consulted databases, taking into account the differences of the various controlled vocabularies as well the differences of database specific technical variations (e.g., the use of quotation marks). Case reports, animal-only studies and conference abstracts were excluded. No restrictions were made on language and publication year. The final search was performed on February 19th, 2018 and repeated on June 17, 2019. All search strings used can be found in the supplementary data.

Study selection process

First, duplicate publications were removed. Titles and abstracts of remaining identified publications were screened for eligibility by 6 reviewers (BdR, EvE, HP, IV, KR, MA) in preassembled pairs. Each reviewer of a pair independently screened and coded an assigned part of the articles 'include', 'unclear' or 'exclude'. Disagreements on inclusion were discussed in the pre-assembled pairs until consensus was reached. Subsequently, potentially relevant publications were independently assessed in full-text by three reviewers (BdR, IV, EvE). In case of multiple publications of a single trial, the first published version was included. Discrepancies on the eligibility of articles were resolved by discussion until consensus was reached. The selection of publications was managed by the Rayyan QCRI web application (Qatura Computing Research Institute, 2016) [30].

Data collection and extraction

Data extraction was independently performed by two reviewers (EvE and IV) using a predefined sheet in Microsoft Excel, Version 15.40. Extracted data were compared and discrepancies were resolved. Data were extracted on four categories following the recommendations of the Cochrane Collaboration; characteristics of the study (i.e., dietary comparison, location, design), the participants (i.e., number of randomized/analyzed participants, sex, mean age, mean body weight, mean BMI), the dietary interventions (i.e., compositions, follow-up time) and the outcomes per arm of the trial [21].

Risk of bias assessment

Two reviewers (EvE and IV) independently assessed the risk of bias for included studies, using the Cochrane 'Risk of bias' tool for randomized controlled trials [24]. This tool involved a classification of six different domains of bias (i.e., selection bias, performance bias, attrition bias, detection bias, reporting bias and (design-specific) other sources of bias) with seven corresponding domains: random sequence generation, allocation

concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and "other sources of bias". For detection of the "other sources of bias", reviewers were in particular alert to (self)reporting bias, compliance assessment and carry-over effects in cross-over trials, with trials lacking a wash-out period being at higher risk. Each domain was separately judged as having a "low", "high" or "unclear" risk of bias. In addition, a support for judgement was given and summarised following the criteria outlined by the Cochrane Collaboration [21]. Any discrepancies in bias coding were resolved by discussion.

Direct pairwise meta-analyses

To perform meta-analyses for continuous outcomes measured with different measuring instruments of liver fat on different scales (i.e., MRS/MRI (%) and CT-scans (Hounsfield Units)), effect estimates were expressed as standardized mean difference (SMD) with corresponding 95% confidence interval (95% CI). When studies only reported relative changes in liver fat, the absolute change based on the relative change and the baseline value was calculated. If trials presented medians and interquartile ranges (IQRs), values were converted into means and standard deviations according to the Cochrane Collaboration [24].

Intervention effects were pooled by performing standard pairwise meta-analyses for all comparisons that contained at least three comparisons between diets. A random-effects model was used (method of DerSimonian and Laird [31]) for the comparison between a low-carbohydrate high-fat and a high-carbohydrate low-fat diet and due to the limited number of included studies a fixed-effect model for the other two comparisons. For the study of Luukkonen et al.[32], two interventions (saturated fat and unsaturated fat) were compared against the same control group (carbohydrates). To correct for these multiple correlated comparisons the number of participants in the control arm was divided by the number of comparisons (i.e. two) thereby creating two (reasonably independent) comparisons (Cochrane handbook Chapter 16.5.4). We performed a sensitivity analysis in which the two groups with physical activity as a co-intervention from the study of Bozzetto et al were excluded to eliminate the potential effect of physical activity on the results. The diet that was expected to be beneficial, as described in the rationale of the included studies, was considered as the intervention arm (high unsaturated fat-low saturated fat, high protein-low carbohydrates and high-carbohydrates low-fat), and the other the control arm (saturated fat, high carbohydrates and high fat). As a result, a negative standardized mean difference can be interpreted as a decrease in liver fat in the intervention arm compared with the control arm, which means that the intervention arm is favoured. In case of an overfeeding design, a negative standardized mean difference represents a smaller increase in liver fat in the intervention arm compared to the control arm. A positive standardized mean difference indicates that the control arm is favoured. Guidelines state that an SMD of 0.2 can be considered small, 0.5 as medium and 0.8 as high [33].

Statistical heterogeneity was assessed using the I-squared statistic [37]. Heterogeneity was considered to be low if the I^2 value was under 40%, moderate if between 30% to 60%, substantial if between 50% to 90% and considerable when between 75% and 100% [24]. All statistical analyses were conducted using Stata statistical Software (Statacorp, College Station, Texas, USA) version 14.

Handling missing data

In case of unreported or incomplete data on mean changes (or SD) in liver fat content between baseline and follow-up, the original investigators were contacted and asked to provide missing data. When no response was received, we calculated mean differences using standard deviations based on the information that was provided (baseline or follow-up value with corresponding SD), as described in a previous meta-analysis [34]. Trials were not included when relevant data to calculate mean differences was not provided [21].

Small-study effects

A funnel plot was used for graphical examination of small-study effects [39, 40]. In addition, Egger's test was performed [24, 40] if more than 10 studies for a specific analysis were available [41].

RESULTS

Study selection

Of the 4.291 publications retrieved, a total of 3.320 unique publications were screened on title and abstract (Figure 1). Of those, 3.215 publications were excluded after screening of titles and abstracts for eligibility. A total of 105 articles were assessed for eligibility based on full text, of which 84 were excluded due to the following reasons: no dietary intervention (n=23), interventions not isocaloric (n=10), multiple publications from a single trial (n=4), no original research paper (n=7), co-interventions not equal in both arms (n=2), no adequate comparison (n=3), no MRI/MRS/CT/biopsy liver fat outcome (n=24), population younger than 18 years (n=3) or no RCT design (n=8), leaving a total of 21 included articles [32, 35-54] (**Figure 1**).

For one study, only two out of three arms were incorporated into the meta-analysis, as the diet in one arm contained less calories than the diet in the other two arms [49]. Ultimately, 25 eligible comparisons remained for analyses as three studies contained more than one comparison [32, 38, 39].

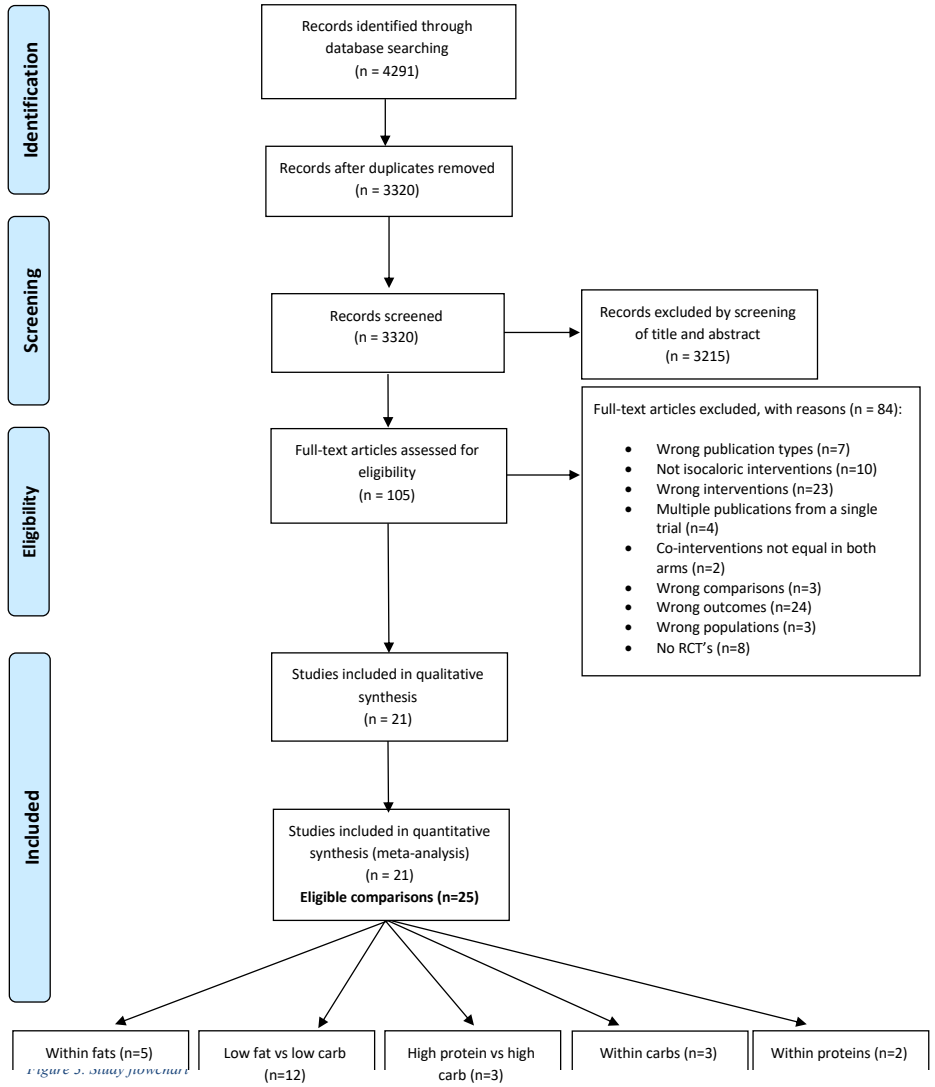

Figure 1. Flowchart of included randomized controlled trials in meta-analysis on dietary macronutrient composition in relation to liver fat

Study characteristics

Table 1 shows the characteristics of the 21 randomized controlled trials. Studies were published between 2002 and 2019 and the number of participants ranged from 7 to 166. The duration of the studies varied between 7 days and two years. With regard to the macronutrient comparisons, ten studies reported effects of a low-carbohydrate high-fat (LCHF)-diet compared with a high-carbohydrate low-fat (HCLF)-diet [32, 38-43, 51-53]. Three studies compared a low-protein high-carbohydrate (LPHC)-diet with a high-protein low-carbohydrate (HPLC)-diet [45, 48, 49]. There were no studies on the comparisons between fat and protein content of the diet.

The other studies performed comparisons between types of macronutrients. A total of five studies compared different types of dietary fat, of which three studies compared a diet high in saturated fatty acids (SFAs) with a diet high in unsaturated fatty acids (UFAs) [32, 37, 50], one study compared trans fatty acids with palm- and sunflower oil [36] and one study looked at replacement of long chain fatty acids with medium chain fatty acids [47]. In two studies dietary fibres were compared with other carbohydrates [35, 39], one study compared whole grain wheats with refined wheats [54] and in two studies diets containing animal protein was compared with diets containing plant/soy protein [44, 46].

In total, sixteen studies used a parallel design, whereas five had a cross-over design [35, 43, 46, 49, 52]. Two studies assessed the liver fat content using CT [47, 48], whereas all other studies used MRS/MRI. One study assessed liver fat content both with MRI and MRS, of which we chose to use the MRS results in the meta-analysis as this is considered the most reliable method [11]. Most studies mainly included participants with overweight or obesity, varying from adolescents to elderly, except for six studies that included lean participants [35, 43, 45, 47, 49, 50](Table 1). The amount of (macro)nutrients exchanged varies considerably between studies (**Supplemental table 1**). Additional information on the macronutrient composition per study arm can be found in Supplemental table 1.

Risk of bias

The risk of bias assessment for included studies can be found in **Table 2**. In six studies there was high risk of performance bias, in two studies there was high risk of detection bias, in four studies of attrition bias, in seven studies of reporting bias and in six studies there was a high risk of other bias.

The majority of the studies had an unclear risk of selection bias due to a lack of information on concealment of allocation. Overall, there was unclear risk of selection bias and detection bias, and substantial risk of performance, attrition, reporting and other types of bias.

Table 1. Characteristics of randomized controlled trials included in meta-analysis on association between dietary macronutrient composition and hepatic triglyceride content

Author, year	Study design	Length (days)	Run-in/ wash-out	Liver fat measure-ment	Men (%)	Age range or mean age (y)	BMI range or mean at baseline (kg/m²)	Intervention	N	Control	N
Bawden, 2016	Cross-over	7	No/Yes	^{1}H-MRS	100	20.1	23.0	LGI (high fiber)	7	HGI (low fiber)	7
Bendsen, 2011	Parallel	112	No/NA	^{1}H-MRS	0	45-70	25-32	Control	23	Trans fatty acids	23
Bjermo, 2012	Parallel	70	No/NA	^{1}H-MRS	34	30-65	30.8[1]	PUFA	28	SFA	28
Bozzetto, 2012[a]	Parallel	56	Yes/NA	^{1}H-MRS	75.0	35-70	29.1[1]	CHO/fiber	9	MUFA	8
Bozzetto, 2012[b]							30.5[1]	CHO/fiber (+ exercise)	10	MUFA (+ exercise)	9
Errazuriz, 2017	Parallel	84	Yes/NA	^{1}H-MRS	53.3	61.7	31.7[1]	Control	11	MUFA	15
Errazuriz, 2017								Fiber	13	Control	11
Haufe, 2017	Parallel	~180	No/NA	^{1}H-MRS	17.6		>25	Low fat	50	Low carbohydrates	52
Gepner, 2019	Parallel	~180	No/NA	MRI	85.4	47.7	30.9[1]	Low fat	79	Mediterranean/low carbohydrate	78
Herpen, 2011	Parallel	42	Yes/NA	^{1}H-MRS	100	55.2	28.8[1]	Low fat/high carbohydrates	9	High fat/ low carbohydates	9
Kirk, 2009	Parallel	42	No/NA	^{1}H-MRS	18.2	43.6	36.5	High carbohydrates	11	Low carbohydrates	11
Luukkonen, 2018	Parallel	21	No/NA	^{1}H-MRS	44.7	48.0	31.0	Carbohydrates	12	Unsaturated fat	12
Luukkonen, 2018								Carbohydrates	12	Saturated fat	14
Luukkonen, 2018								Unsaturated fat	12	Saturated fat	14
Marina, 2014	Cross-over	28	Yes/Yes	^{1}H-MRS	76.9	36.0	33.6	Low fat	10	High fat	10
Markova, 2017	Parallel	42	No/NA	^{1}H-MRS	64.9	49-78	30.2[1]	Animal protein	18	Plant protein	19
Martens, 2014	Parallel	84	No/NA	^{1}H-MRS	33.3	24.0	22.9	High protein/low carbohydates	7	Low protein/high carbohydrates	9
Nosaka, 2002	Parallel	28	No/NA	CT	100	27-51	23.1[1]	Long-chain triacylglycerols	11	Medium-chain triacylglycerol	11
Ooi, 2015	Parallel	~730	No/NA	CT	0	70-80	26.5[1]	Protein	82	Control	84
Rietman, 2014	Cross-over	14	Yes/No	^{1}H-MRS	70.4	22.8	21.5	Normal protein/ normal carbohydrates	17	High protein/ low carbohydrates	17
Rosqvist, 2014	Parallel	49	No/NA	MRI	70.3	20-38	20.3[1]	SFA	19	PUFA	18
Schutte, 2018	Parallel	84	Yes/NA	1H-MRS	62.0	45-70	27.81	Whole grain wheat	20	Refined wheats	18
Utzschneider, 2013	Parallel	28	No/NA	^{1}H-MRS	37.0	69.3	27.4[1]	Low SAT/LGI	20	High fat/HGI	15
Van Nielen, 2014	Cross-over	28	Yes/NA	^{1}H-MRS	0	61.0		Soy protein	10	Mixed protein	10
Westerbacka, 2005	Cross-over	14	Yes/Unclear	^{1}H-MRS	0	43	33.0	Low fat	10	High fat	10

[1]Weighted mean BMI based on mean baseline BMI values of separate arms. BMI, body mass index; CHO, carbohydrates; CT, computed tomography; ^{1}H-MRS, proton magnetic resonance spectroscopy; HGI, high glycaemic index; LGI, low glycaemic index; MRI, magnetic resonance imaging; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids; SFA, saturated fatty acids.

Table 2. Risk of bias of randomized controlled trials included in systematic review and meta-analysis on macronutrient and macronutrient types composition in relation to liver fat content in adults of 18 years and older

	Selection bias	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
First author	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias
Bawden, 2017	Unclear	Unclear	Unclear	Low	Unclear	Unclear	Low
Bendsen, 2011	Low	Low	Low	Low	Low	Unclear	Unclear
Bjermo, 2012	Unclear	Unclear	High	Low	High	Unclear	High
Bozzetto, 2012	Low	Low	Unclear	Low	Low	Unclear	High
Errazuriz, 2007	Unclear	Unclear	Unclear	Low	Low	High	Low
Haufe, 2017	Low	Low	High	Unclear	High	Unclear	Unclear
Gepner, 2019	Unclear	Unclear	High	Low	High	Unclear	High
Herpen, 2011	Low	Unclear	Unclear	Unclear	Low	High	High
Kirk, 2009	Unclear	Unclear	Unclear	Unclear	Low	Unclear	High
Luukkonen, 2018	Unclear	Unclear	High	Low	Low	Low	Low
Marina, 2014	Unclear	Unclear	Unclear	Low	High	High	High
Markova, 2017	Low	Unclear	High	High	Unclear	Low	Unclear
Martens, 2014	Low	Low	High	Unclear	High	High	Low
van Nielen, 2014	Unclear	Unclear	High	Low	Unclear	Low	Low
Nosaka, 2002	Low	Unclear	Low	Unclear	Low	Unclear	Unclear
Ooi, 2015	Low	Low	Low	Low	Low	High	Low
Rietman, 2014	Low	Low	Low	Unclear	Low	High	Unclear
Rosqvist, 2014	Low	Unclear	Low	Low	Unclear	Unclear	Unclear
Schutte, 2018	Unclear	Unclear	Low	Unclear	High	Unclear	Low
Utzschneider, 2012	Unclear	Unclear	Low	Low	Low	Unclear	Unclear

Effects of interventions

Table 3 provides a summary of findings for all included trials. It also shows the changes in liver fat content and corresponding SMDs for all studies individually. Based on all included trials, we were able to perform three meta-analyses, as described below. A total of 21 studies were included, comprising a total of 25 comparisons between different diets. As we decided to only perform a meta-analysis on exchanges that contained at least three comparisons between dietary intervention arms, we could not meta-analyse comparisons of trans fats with palm- and sunflower oil, long chain with medium chain fat, dietary fibre with other carbohydrates, whole grain wheats with refined wheats, and animal protein with plant protein. Due to the limited number of included trials, we were not able to perform subgroup analyses on disease state, sex, ethnicity or study duration. Moreover, as there were no studies comparing dietary protein with fat, we could not

perform a network meta-analysis in which all macronutrients could be compared both directly and indirectly [35, 36, 39, 44, 46, 47].

High-carbohydrate low-fat versus low-carbohydrate high-fat diets

Out of 12 comparisons for a low-carbohydrate high-fat with a high-carbohydrate low-fat diet, three comparisons favoured a low-carbohydrate high-fat diet over a high-carbohydrate low-fat diet [38, 39], while two other comparisons showed the opposite [41, 52] **(Figure 2).** The other studies showed no difference. Heterogeneity was substantial (67.8%). No small study effects seemed to be present (**Supplemental figure 1**) (*P*-value for Egger's test 0.58). The overall pooled effect of high-carbohydrate low-fat versus high-fat low-carbohydrate was: SMD 0.01, 95% CI -0.36; 0.37 (Figure 2).

After excluding the two groups with a co-intervention of physical exercise from the study of Bozzetto, results were similar (data not shown).

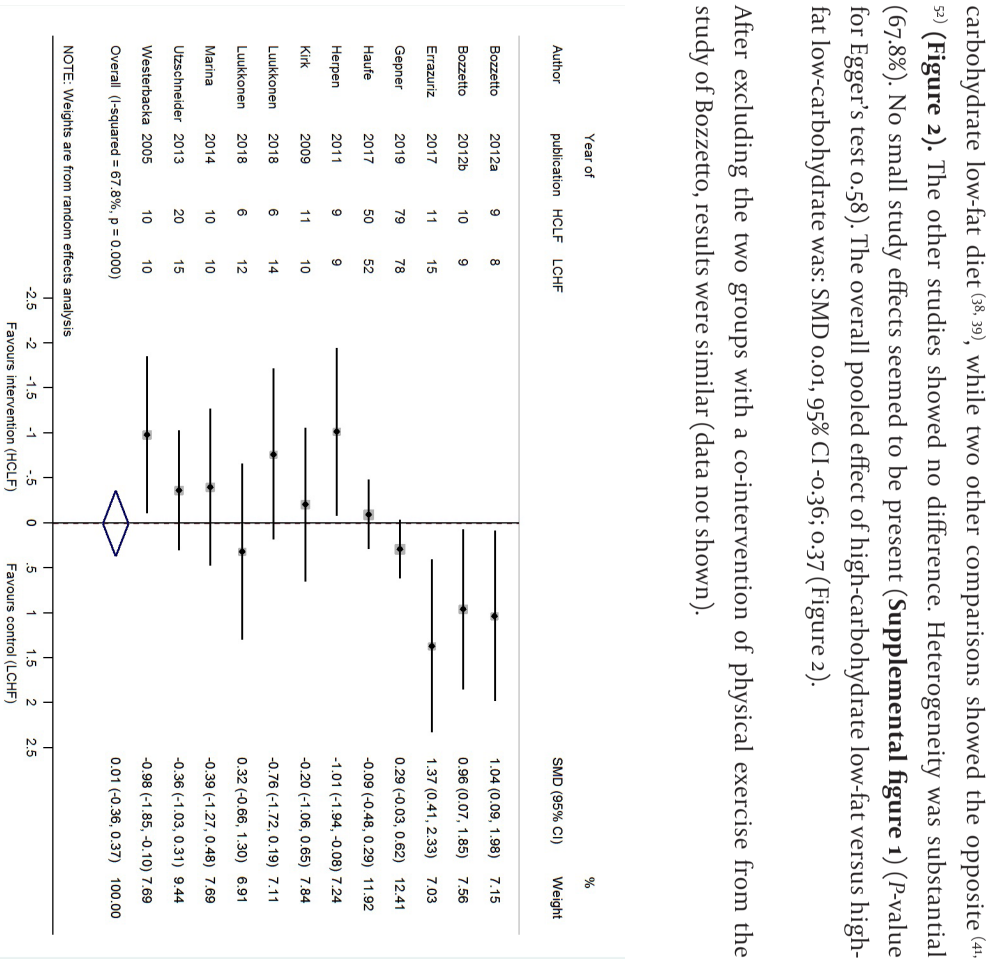

Figure 2. Difference between effects of a low-carbohydrate high-fat diet (LCHF) and a high-carbohydrate low-fat (HCLF) on liver fat content in studies included in meta-analysis: a random effects model. Standardized mean difference (SMD) was calculated by dividing the mean difference between the arms by the standardized deviation of the difference between the arms. A negative standardized mean difference can be interpreted as a decrease in liver fat in the intervention arm compared with the control arm, which means that the intervention arm is favoured.

Table 3. Standardized mean differences of randomized controlled trials included in meta-analysis on association between dietary macronutrient composition and hepatic triglyceride content

Author	Intervention	N	Change in liver fat after intervention (% or HU)	Control	N	Change in liver fat after intervention (% or HU)	Mean difference in change in liver fat between arms (intervention-control, % or HU)	Standard deviation of mean difference	Standardized mean difference
Bawden, 2016	Fibre	7	-0.4	Other carbs	7	1.3	-1.70	1.46	-1.16
Bendsen, 2011	Palm/sunflower oil	23	-0.6	Trans fatty acids	23	-0.8	0.20	4.10	0.05
Bjermo, 2012	PUFA	28	-0.9	SFA	28	0.3	-1.20	2.01	-0.60
Bozzetto, 2012[a]	Low fat	9	-1.6	Low carb	8	2.2	0.60	0.58	1.04
Bozzetto, 2012[b]	Low fat	10	0.1	Low carb	9	-2.5	2.60	2.70	0.96
Errazuriz, 2017	Low fat	11	0.7	Low carb	15	-1.7	2.40	1.75	1.37
Errazuriz, 2017	Fibre	13	-0.6	Other carbs	11	0.7	-1.30	1.33	-0.98
Gepner, 2019	Low fat	79	-5.8	Low carb	78	-7.3	1.5	5.31	0.29
Haufe, 2017	Low fat	50	-4.0	Low carb	52	-3.6	-0.40	4.31	-0.09
Herpen, 2011	Low fat	9	-0.52	Low carb	9	0.37	-0.89	0.88	-1.01
Kirk, 2009	Low fat	11	-4.98	Low carb	10	-4.71	-0.27	1.35	-0.20
Luukkonen, 2018	UFA	12	0.79	SFA	14	2.72	-1.93	1.76	-1.10
Luukkonen, 2018	Low fat	12	1.37	Low carb	14	2.72	-1.35	1.77	-0.76
Luukkonen, 2018	Low fat	12	1.37	Low carb	12	0.79	0.58	1.78	0.32
Marina, 2014	Low fat	10	-2.2	Low carb	10	-1.3	-0.90	2.28	-0.39
Markova, 2017	Plant protein	17	-6.8	Animal protein	15	-6.7	-0.10	8.96	-0.01
Martens, 2014	High protein	7	-0.03	High carb	9	0.05	-0.08	0.08	-1.05
Nosaka, 2002	Long chain FA	11	0.03	Medium chain FA	11	0.02	0.01	0.10	0.1
Ooi, 2015	High protein	82	0.00	High carb	84	0.04	-0.04	0.20	-0.2
Rietman, 2014	High protein	17	-0.05	High carb	17	0.11	-0.16	0.26	-0.62
Rosqvist, 2014	PUFA	18	0.04	SFA	19	0.56	-0.52	0.71	-0.73
Utzschneider, 2013	Low fat	20	-0.50	Low carb	15	0.4	-0.9	2.50	-0.36
Van Nielen, 2014	Soy protein	10	-0.4	Meat protein	10	-0.9	0.5	0.84	0.59
Schutte, 2018	Whole grain wheats	20	0.53	Refined grain wheats	18	2.00	-1.47	2.00	-0.73
Westerbacka, 2005	Low fat	10	-2.0	Low carb	10	3.5	-5.5	5.62	-0.98

FA, fatty acid; HU, Hounsfield Unit; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids; SFA, saturated fatty acids. Standardized mean difference (SMD) was calculated by dividing the mean difference between the arms by the standardized deviation of the difference between the arms.

Dietary saturated fat versus unsaturated fat

Only three studies examined the effect of unsaturated fat compared to saturated fat, of which all three found that an unsaturated fat diet reduces liver fat compared with saturated fat [32, 37, 50] **(Figure 3).** The overall effect showed that unsaturated fat as compared with saturated fat reduced liver fat to a large extent (SMD -0.75, 95% CI -1.11; -0.39. A funnel plot is shown in **Supplemental figure 2**; Egger's test was not performed due to an insufficient number of included studies.

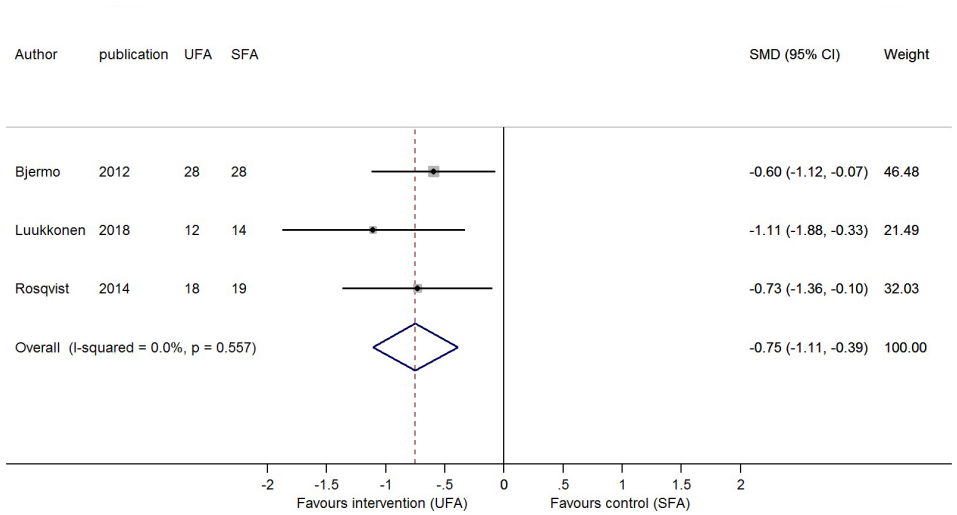

Figure 3. Difference between effects of a diet high in saturated fats (SFA) and a diet high in unsaturated fat (UFA) on liver fat content in studies included in meta-analysis: a fixed effects model. Standardized mean difference (SMD) was calculated by dividing the mean difference between the arms by the standardized deviation of the difference between the arms. A negative standardized mean difference can be interpreted as a decrease in liver fat in the intervention arm compared with the control arm, which means that the intervention arm is favoured.

High-protein low-carbohydrate versus low-protein high-carbohydrate diets

Three studies assessed the effect of a high protein-low carbohydrate compared to a low-protein high-carbohydrate diet on liver fat. One study found that a high-protein low-carbohydrate diet resulted in reduced liver fat content compared to a low-protein high-carbohydrate diet [45], whereas the other two studies did not find a difference [48, 49] (**Figure 4**). The overall pooled effect showed that a high-protein low-carbohydrate diet moderately reduced liver fat as compared to a low-protein high-carbohydrate diet (SMD -0.32, 95%CI -0.58; -0.05). A funnel plot is shown in **Supplemental figure 3**, Egger's test was not performed due to an insufficient number of included studies.

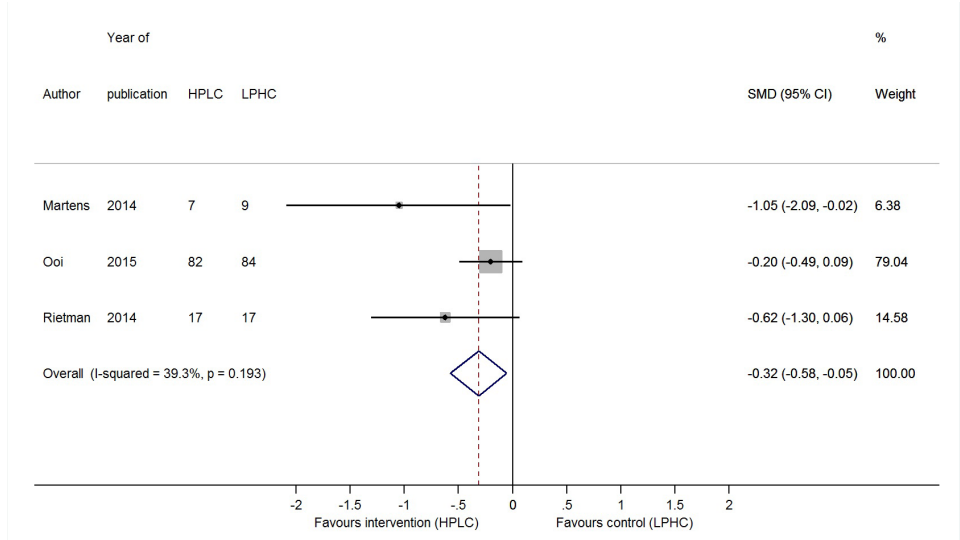

Figure 4. Difference between effects of a low-protein high-carbohydrate (LPHC) diet and a high-protein low-carbohydrate (HPLC) diet on liver fat content in studies included in meta-analysis: a fixed effects model. Standardized mean difference (SMD) was calculated by dividing the mean difference between the arms by the standardized deviation of the difference between the arms. A negative standardized mean difference can be interpreted as a decrease in liver fat in the intervention arm compared with the control arm, which means that the intervention arm is favoured.

DISCUSSION

With this systematic review and meta-analysis including randomized controlled trials we have provided a summary of the evidence on the effect of dietary macronutrient composition on the amount of liver fat, as assessed by ^{1}H-MRS, MRI or CT. Our results show that replacing dietary fat with carbohydrates did not result in changes in liver fat. Diets high in unsaturated fat lead to a larger decrease (or smaller increase in case of an overfeeding design) in liver fat content than diets high in saturated fat. A high-protein low-carbohydrate diet reduces liver fat as compared with a low-protein high-carbohydrate diet.

Our results focusing on liver fat content are in line with the review of Parry and Hodson, in which the authors describe that most studies suggest no influence on liver fat by diets that are high in carbohydrates in the form of free sugars [19]. The increase in liver fat observed in diets high in fat seems to be attributable to an increased saturated fat consumption, while increased consumption of mono- or polyunsaturated fat may reduce liver fat content [19], which supports the results of our meta-analysis. The beneficial effects of unsaturated fat on liver fat content compared to saturated fat were also reported in another recent review [55]. Additionally, results from this meta-analysis are in agreement with the findings from a meta-analysis on the effects of mutual exchanges of different dietary fats and carbohydrates on glucose-insulin homeostasis, an outcome strongly related to NAFL. The authors of this meta-analysis found that replacement of carbohydrates or saturated fat with polyunsaturated fat led to an improved insulin secretion capacity, lower fasting glucose, improved Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and lower haemoglobin A1C (HbA1c)[55]. The exchange of saturated fat for carbohydrates did not affect most outcomes, except for a decrease in fasting insulin [56].

Although the pathogenesis of liver fat accumulation is not completely elucidated yet, it is assumed that both high caloric intake and dietary composition influence liver fat content. Dietary intake of specific nutrients (e.g. fructose) may increase *de novo* lipogenesis, and together with increased lipolysis of visceral fat this may contribute to an increased flux of free fatty acids in the liver, leading to hepatic fat accumulation [10, 57]. Additionally, n-6 polyunsaturated fatty acids have been suggested to suppress lipogenic gene expression and could thereby decrease de novo lipogenesis and thereby decrease accumulation of liver fat [58], which is consistent with the findings of this meta-analysis showing that this holds true more generally for unsaturated fat and that exchanging saturated for unsaturated fat can lower liver fat.

A strength of this study is that it is the first comprehensive meta-analysis on the effect of macronutrient composition and macronutrient types on liver fat. The review process has been performed systematically and only studies in which liver fat was measured with either MRI, ^{1}H-MRS or CT were included. Moreover, we only included studies that performed a dietary intervention rather than only providing dietary advice.

This study also has some limitations. The first one is that comparing and meta-analysing data from different dietary intervention trials appeared challenging, as there was considerable heterogeneity in study duration and composition of the diets, percentages of macronutrients exchanged, and total amount of energy of provided diets (hyper-, hypo- or isocaloric). Firstly, whereas some studies specified which subtypes of dietary fats or carbohydrates were replaced, others did not, making the interpretation of the results difficult. As our results on exchanging unsaturated with saturated fat have shown, the fat type that is replacing the carbohydrates is likely relevant. Three randomized trials [32, 38, 39] replaced carbohydrates with unsaturated fats and show that a low-carbohydrate high-fat diet leads to less liver fat compared with a high-carbohydrate low-fat diet, whereas most other studies suggest that a high-carbohydrate low-fat diet leads to less liver fat. However, information on the type of fat used to replace carbohydrates in most studies lacking.

Secondly, this meta-analysis focused on the exchange between two macronutrient (subtypes) irrespective of the energy percentage derived from these specific macronutrients. Therefore, the studies show marked heterogeneity in the percentual energy contribution of the macronutrient subtypes that were exchanged. Studies with a larger exchanged energy percentage of macronutrients between the compared diets may have resulted in larger effect estimates than studies with smaller exchanges in energy percentages. However, the effect sizes of the studies were not proportional to the amount of energy percentage that was exchanged.

Thirdly, total caloric intake varied considerably between studies. Whereas some studies used an overfeeding design in which participants were instructed to consume more calories than their usual diet, other studies used an isocaloric or hypocaloric diet. Our only criterion regarding energy intake was that it should be equal in both study arms within a trial, regardless of whether energy intake was below, above or equal to the energy requirement of the participants. Therefore, mean caloric intake varied from 1.100 kilocalories per day [42] to over 3.400 kilocalories per day [49]. Although the number of included arms was too small to perform stratified analyses, the effect of macronutrient composition did not seem to be modified by caloric intake after visual inspection in the meta-analysis on dietary carbohydrates versus fat, which included the most comparisons. A second limitation of this review is that data of variance within the dietary arms of the included trials (e.g. variance of mean change in liver fat or variance of mean difference) were not always reported. Therefore, P-values of the mean differences in change in liver fat – that were converted to corresponding t-values – had to be used to calculate the standard deviations, standard error of the means and the 95% CIs of the mean differences in change in liver fat by Cochrane equations [59]. With these calculated values, mean differences could be converted to standardized mean differences and their corresponding 95% CIs. However, some studies did not present exact P-values of the mean difference, but exclusively presented the level of significance (e.g., $P < 0.05$ or $P < 0.01$). As

described by the Cochrane Handbook, the limits of the significance level were used for these trials as a conservative approach [59]. This approach may have caused imprecision of the variance for each trial, which is reflected in a larger confidence interval around the SMD and a decreased weight of the study [59].

As only a limited number of studies could be included in this meta-analysis, we recommend that more large randomized controlled dietary trials with a low risk of bias and of sufficient power are performed, in which complete and transparent reporting of results is of great importance in order to address this gap in knowledge. Especially trials in which proteins and fats are exchanged are warranted, as they were completely lacking, and then preferably with three arms to compare carbohydrates, fats and proteins in one study in which the sources and types of these macronutrients are specified. Bridging this gap in research is essential for the development of preventive strategies for fatty liver in the future.

In conclusion, this systematic review and meta-analysis of randomized controlled trials showed that replacing total carbohydrates with total fats has no effect on liver fat content. Replacing saturated fat with unsaturated fat resulted in a decrease or a smaller increase in liver fat content, and replacing carbohydrates with proteins also seems to lead to less liver fat. Only a limited number of eligible studies could be included, which supports an essential need for additional experimental studies on dietary macronutrient composition and liver fat content in order to provide optimal prevention and treatment for non-alcoholic fatty liver by dietary interventions.

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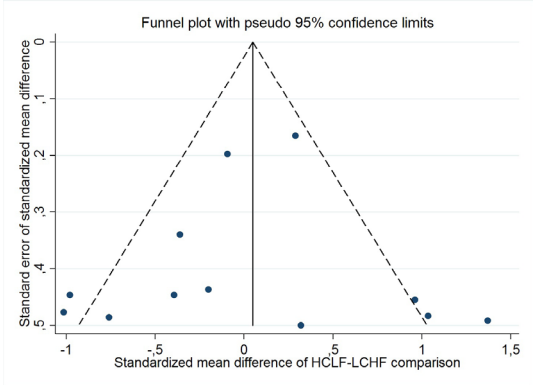
SUPPLEMENTARY FILES

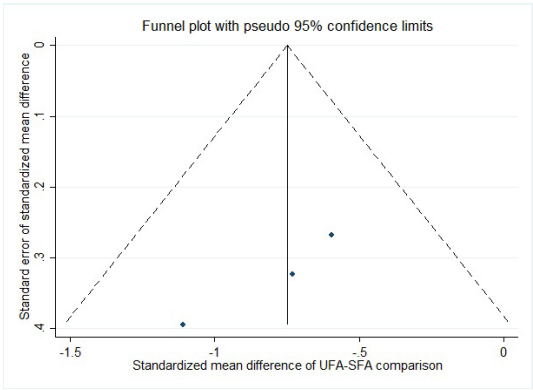
Supplemental table 1. Macronutrient composition per arm of randomized controlled trials included in meta-analysis on association between dietary macronutrient composition and hepatic triglyceride content

Author	Arm	%En (CHO/fat/protein)	Mean caloric intake (kcal)	Arm	%En (CHO/fat/protein)	Mean caloric intake of range (kcal)
Bawden, 2016	Fibre	71/14/14	2004	Other carbs	71/14/14	2003
Bendsen, 2011	Trans fatty acids	44.2/37.1/14.5	1982	Palm/ sunflower oil	44.0/33.9/16.0	1913
Bjermo, 2012	PUFA		2190	SFA		2170
Bozzetto, 2012	Low fat	53/28/19	1873	Low carb	40/42/18	2039
Bozzetto, 2012 (+ exercise)	Low fat	53/29/18	2037	Low carb	40/42/18	2480
Errazuriz, 2017	Low fat	–/34/17	2006	Low carb	–/46/14	2064
	Fibre	–/28/17	1889			
Gepner, 2019	Low fat		2852	Low carb		2839
Haufe, 2017	Low fat			Low carb		
Herpen, 2011	Low fat	56.0/21.7/16.3	2169	Low carb	34.0/49.3/15.2	2345
Kirk, 2009	Low fat	65/20/15	1100	Low carb	10/75/15	1100
Luukkonen, 2018	PUFA/MUFA	22.7/59.7/13.2	2883	SFA	25.9/58.9/15	2787
	Low fat	63.7/23.8/11.4	2902			
Marina, 2014	Low fat	61.7/20.2/18.1	3321	Low carb	27.4/54.8/17.8	3208
Markova, 2017	Plant protein	39.2/30.9/29.9		Animal protein	40.4/30.1/29.5	
Martens, 2014	High protein	35/35/30		High carb	60/35/0	
Van Nielen, 2014	High protein soy	49/27/22	2174	High protein no soy	52/26/21	2150
Nosaka, 2002	Long chain FA	58.4/27.0/12.8	2330	Medium chain FA	57.9/27.2/12.8	2320
Ooi, 2015	High protein	41.0/31.0/23.0	1757	High carb	46.0/31.0/18.0	1717
Rietman, 2014	High protein	36.6/37.7/25.7	3439	High carb	45.2/39.4/15.4	3463
Rosqvist, 2014	PUFA	43.3/40.3/11.8	3136	SFA	47.7/36.8/11.5	3035
Utzschneider, 2013	Low fat	57.3/23.0/17.3	2241	Low carb	37.9/43.0/16.4	2354
Westerbacka, 2005	Low fat	61/16/19		Low carb	31/56/13	

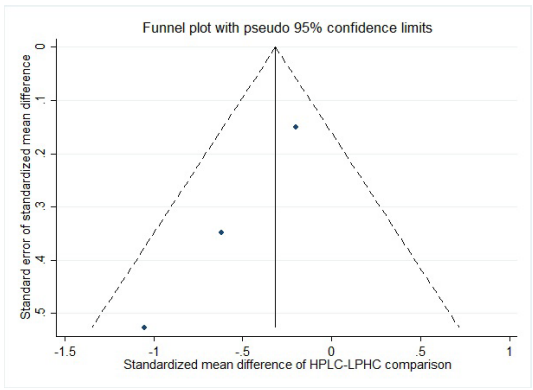
CHO, carbohydrates; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids; SFA, saturated fatty acids.



Supplemental figure 1. Funnel plot of studies comparing a low-carbohydrate high-fat (LCHF) diet and a high-carbohydrate low-fat (HCLF) diet



Supplemental figure 2. Funnel plot of studies comparing a unsaturated fat (UFA) diet and a saturated fat (SFA) diet



Supplemental figure 3. Funnel plot of studies comparison a high-protein low-carbohydrate (HPLC) diet and a low-protein high-carbohydrate (LPHC) diet

3

Sweet Snacks Are Positively and Fruits and Vegetables Are Negatively Associated with Visceral or Liver Fat Content in Middle-Aged Men and Women

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ABSTRACT

Background
Visceral adipose tissue (VAT) and hepatic triglyceride content (HTGC) are major risk factors for cardiometabolic diseases.

Objective
We aimed to investigate the association of dietary intake of the main food groups with VAT and HTGC in middle-aged men and women.

Methods
We used data from the Netherlands Epidemiology of Obesity Study, a population-based study including 6,671 participants aged 45-65 years at baseline. In this cross-sectional analysis, VAT and HTGC were assessed by magnetic resonance imaging and spectroscopy, respectively, as the primary outcomes. Habitual intake of main food groups (dairy, meat, fish, fruits and vegetables, sweet snacks, and fats and oils) was estimated using a food frequency questionnaire. We examined associations of intake of different food groups with VAT and HTGC by linear regression analysis stratified by sex and adjusted for age, smoking, education, ethnicity, physical activity, basal metabolic rate, energy-restricted diet, menopausal state and total energy intake, stratified by sex.

Results
In women, a 100-g/d higher intake of dairy was associated with 2.0 cm^2 less VAT (95% CI: −3.4, −0.7 cm^2) and a 0.95-fold lower HTGC (95% CI: 0.90-, 0.99-fold). Moreover, a 100-g/d higher intake of fruit and vegetables was associated with 1.6 cm2 less VAT (95% CI: −2.9, −0.2 cm2) in women. Fruit and vegetables were negatively associated (0.95; 95% CI: 0.91, 1.00) with HTGC, and sweet snacks were positively associated (1.29; 95% CI: 1.03, 1.63). Patterns were weaker but similar in men. Fish intake was not associated with VAT or HTGC and plant-based fat and oil intake were only associated with VAT after adjustment for total body fat.

Conclusions
Despite some variation in the strength of the associations between men and women, dietary intake of sweet snacks was positively associated with HTGC, and fruit and vegetable intake were inversely associated with visceral fat and liver fat content. Prospective studies are needed to confirm these results.

INTRODUCTION

Obesity, in particular abdominal obesity, is increasingly prevalent worldwide and is a major risk factor for type 2 diabetes and cardiovascular diseases [1, 2]. The excess cardiometabolic risk associated with abdominal obesity is hypothesized to be due to the accumulation of fat in non-adipose tissue [2]. Visceral adipose tissue (VAT) and hepatic triglyceride content (HTGC) have been associated with a cluster of metabolic risk factors, insulin resistance, coronary artery disease and cardiovascular disease in general [3-6]. Furthermore, visceral adipose tissue is thought to possibly contribute to the excess cardiometabolic risk due to a high free fatty acid (FFA) release and a high rate of cytokine secretion [2]. In addition, high concentrations of free fatty acids and insulin resistance are related to fat deposition in the liver [7] and are strongly related to type 2 diabetes and cardiovascular disease [8, 9].

Due to the many health-related consequences, both visceral fat and liver fat might be a key targets in the prevention or treatment of cardiometabolic disease and its consequences. In addition to physical activity, diet is a key modifiable lifestyle risk factor for obesity and chronic diseases [10-12]. A recent systematic review has shown that dietary patterns recognized as healthy and intake of medium-chain triacylglycerols (MCTG) display an inverse association with visceral and subcutaneous fat. For visceral fat only, inverse associations were also shown with dietary fiber, calcium and phytochemicals [13]. Interestingly, an overfeeding study of saturated and polyunsaturated fat showed distinct effects on visceral and liver fat [11]. Most previous studies have assessed the role of nutrients in fat deposition [14-18], although higher energy intake during childhood has been suggested to be associated with greater NAFLD risk irrespective of the macronutrients this energy intake comes from [19]. However, it is increasingly recognized that studying foods and food groups rather than single nutrients may be important in relation to health outcomes, as foods are not merely the sum of their nutrients [20-23]. Within a food item, there may be unknown effects of other nutrients, or interactions between the separate nutrients, and the food matrix may play a role [22-24]. Countries throughout Europe as well as the United States now have published dietary guidelines based on whole food products and groups rather than single nutrients [25]. While evidence on major food groups (e.g. dairy, meat, fruit and vegetables) in relation to body weight [26] and clinical cardiometabolic outcomes including CHD [27] and diabetes [28] is increasing, knowledge of the relationships between food groups and ectopic fat deposition is scarce. We therefore aimed to investigate the associations between dietary intake of the main food groups and visceral fat and liver fat content in a population-based cohort of middle-aged men and women.

SUBJECTS AND METHODS

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study in 6,671 individuals aged 45 to 65 years, with an oversampling of persons with a BMI of 27 kg/m^2 or higher. Detailed information about the study design and data collection has been described elsewhere [29]. Men and women aged between 45 and 65 years with a self-reported BMI of 27 kg/m^2 or higher living in the greater area of Leiden (in the west of The Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI, allowing a reference distribution of BMI.

Participants visited the NEO study center of the Leiden University Medical Center (LUMC) after an overnight fast. Prior to the NEO study visit, participants completed a questionnaire about demographic and clinical information, as well as a food frequency questionnaire. At the study center, the participants completed a screening form asking about anything that might create a health risk or interfere with magnetic resonance imaging (MRI) (most notably metallic devices, claustrophobia, or a body circumference of more than 1.70 m). Of the participants who were eligible for MRI, approximately 35% were randomly selected to undergo direct assessment of abdominal fat.

The present study is a cross-sectional analysis of the baseline measurements of the participants with a measurement of visceral adipose tissue. We excluded participants with self-reported diabetes prior to the study visit, participants with missing data on dietary intake, participants with implausibly high or low total energy intake (<600 kcal/day or >5,000 kcal/day) or participants with missing data on potential confounding factors. For the analyses on hepatic triglyceride content, we additionally excluded participants without assessment of hepatic triglyceride content and those who consumed more than four units of alcoholic beverages per day.

The study was approved by the medical ethics committee of the Leiden University Medical Center and all participants gave written informed consent.

Data collection

On the questionnaire, participants reported ethnicity by self-identification in eight categories which we grouped into white (reference) and other ethnicity. Tobacco smoking was categorized as current, former, or never smoking (reference). The highest level of education was reported in 10 categories according to the Dutch education system and grouped into high (including higher vocational school, university, and postgraduate education) versus low education (reference). Participants reported their medical history of diabetes and cardiovascular diseases. Body weight and percent body fat were assessed by the Tanita bio impedance balance (TBF-310, Tanita International Division, UK) without shoes, and one kilogram was subtracted from the body weight. BMI was calculated by dividing the weight in kilograms by the height in meters squared. The menopausal state was categorized as pre-, or postmenopausal according to information on ovariectomy, hysterectomy and the self-reported state of menopause in the questionnaire. The basal metabolic rate was calculated based on age, sex, height and weight according to the Mifflin-St Jeor equation. Participants reported the frequency and duration of their physical activity during leisure time, which was expressed in hours per week of metabolic equivalents (MET h/week) [30].

Dietary intake of food groups

Habitual dietary intake of all participants was estimated using a self-administered, semiquantitative 125-item food frequency questionnaire (FFQ) [31, 32]. In this FFQ, participants were asked about the frequency of intake of foods during the past month (times per day, week, month, never). Additionally, the serving size was estimated (spoons of potatoes, pieces of fruit). In a random subsample of 110 men and 119 women, the relative validity of the FFQ against two 24-h dietary recalls was assessed regarding total fatty acids, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). The correlation coefficients corrected for within-person variation for total fatty acids, SFAs, MUFAs and PUFAs were approximately 0.5 [33]. Intake of nutrients and total energy was calculated using the Dutch Food Composition Table (NEVO-2011).

Based on the FFQ, products were categorized into food groups on the basis of similar source, nutrient characteristics or hypothesized biological effects [28]. Hereby, we followed the categorization of food groups of the Netherlands Nutrition Center [34] as much as possible based on the distinctive capabilities of the FFQ used. Food groups were categorized into dairy (including milk, cheese, yogurt and butter), meat, fruits and vegetables, sweet snacks (chocolate, cake, pie, candy bars and candy), fish and plant-based fats and oils (margarine, cooking oils). Additionally, subdivisions were made: dairy was also subdivided into cheese, milk, butter and yogurt; fruit and vegetables were studied separately; fats and oils were divided into margarine and oils; and sweet snacks were divided into cake and candy. The caloric intake of products within these food groups was summed and converted into percent of total energy intake (En%) by dividing the caloric intake of the food groups by the total caloric intake per day.

Visceral adipose tissue and hepatic triglyceride content assessment by imaging techniques

Imaging was performed on a 1.5 Tesla MR system (Philips Medical Systems, Best, The Netherlands). Visceral adipose tissue was quantified by a turbo spin echo imaging protocol using MRI. At the level of the fifth lumbar vertebra, three transverse images each with a slice thickness of 10 mm were obtained during a breath-hold. Visceral fat area was converted from the number of pixels to centimeters squared using in-house-developed software (MASS, Medis, Leiden, The Netherlands) and the average of the three slices was used in the analyses [29].

Hepatic triglyceride content was quantified by proton spectroscopy (^{1}H-MRS) of the liver [35]. An 8 ml voxel was positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Sixty-four averages were collected with water suppression. Spectra were obtained with an echo time of 26 ms and a repetition time of 3,000 ms. Data points (1,024) were collected using a 1,000 Hz spectral line. Without changing any parameters, spectra without water suppression, with a repetition time of 10 s and four averages were obtained as an internal reference. ^{1}H-MRS data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium), as described previously [36]. Hepatic triglyceride content relative to water was calculated as the sum of the signal amplitudes of methyl and methylene divided by the signal amplitude of water and then multiplied by 100.

Statistical analysis

In the NEO study, there is an oversampling of persons with a BMI of 27 kg/m^2 or higher. To correctly represent associations in the general population [37], adjustments for the oversampling of individuals with a BMI ≥ 27 kg/m^2 were made by weighting individuals toward the BMI distribution of participants from the Leiderdorp municipality [38], which was similar to that of the general Dutch population [39]. All results were based on the weighted analyses, and consequently, the results apply to a population-based study without oversampling of individuals with a BMI ≥ 27 kg/m^2. As a result of the weighted analyses, percentages and proportions are given instead of numbers of participants. Other characteristics are expressed as percentages or means (standard deviations). We tested for interactions with sex and performed all analyses for the total population and for men and women separately due to major differences in body fat distribution between men and women and previously observed gender differences in the relation between food group scores and abdominal obesity [40]. Linearity of the main food groups was checked by adding a quadratic term to the main multivariable adjusted model and visual inspection of scatter plots.

We performed linear regression analyses with multiple models to examine the associations between dietary intake of the food groups with visceral fat and liver fat content. First, we examined the crude associations of dietary intake of 100 grams/day of each food group with visceral fat and liver fat content. Second, we adjusted the models for age, smoking, education, ethnicity, physical activity, basal metabolic rate, menopausal state, total energy intake and adherence to an energy restricted diet and liver fat models were also adjusted for alcohol intake. Third, we additionally adjusted the models for total body fat, to examine whether the associations were specific for visceral fat and liver fat instead of merely reflecting associations with overall adiposity. Fourth, to examine whether associations were specific for the food groups and not merely reflecting a healthy diet, we additionally adjusted all models for the food group fruit and vegetables, and the food group fruit and vegetables model for the food sweet snacks. As secondary analyses, we subdivided several food groups into a finer categorization: dairy into milk, cheese, yogurt and butter; sweet snacks into cake and candy; plant-based fat and oils into margarine and oils; and fruit and vegetables into fruit and vegetables separately. We performed subgroup analyses and stratified the multivariable model 2 (not including total body fat and markers of a healthy diet) by and menopausal state (pre- and postmenopausal). This stratification was done because for example visceral fat may differ greatly between pre- and postmenopausal women [41]. We additionally stratified the same multivariable models of hepatic triglyceride content by the rs738409 single nucleotide polymorphism in the PNPLA3 gene to examine whether associations would be different in carriers of the risk allele that is associated with high liver fat content [42]. Due to a skewed distribution of hepatic triglyceride content, we used the natural logarithm of this variable in the analyses. For interpretation of the results, we back transformed the regression coefficients of hepatic triglyceride content toward a ratio with 95% confidence interval, which can be interpreted as a ratio in hepatic triglyceride content associated with dietary intake of 100 grams/day of the food groups (for example 1.2, can be interpreted as a 1.2-fold higher hepatic triglyceride content, which in a person with a hepatic triglyceride content of 5% would reflect an increase to 6%). The regression coefficients of visceral adipose tissue represent an absolute difference in visceral adipose tissue in cm^2 per 100 grams/day of the food groups.

As a means of sensitivity analysis, we additionally performed two types of isocaloric substitution analyses in which dairy was specifically replaced by the other food groups: one per 100 grams per day and one per 10% of the energy (En%) derived from the food groups.

In these substitution models, we included all the food groups under study (meat, fruits and vegetables, sweet snacks, fish, and plant-based fats and oils), except dairy, the food

group to be substituted, in addition to all other food consumed that was not categorized in one of the food groups, and all confounding factors. Finally, we performed a sensitivity analysis including a variable in our fully adjusted model that divides energy intake by basal metabolic rate, to adjust for potential under- and overreporting.

We performed all analyses using STATA statistical software (Statacorp, College Station, Texas, USA), version 14.

RESULTS

In total, 6,671 participants were included in the NEO study between September 2008 and October 2012, of whom 2,580 underwent an MRI of the abdomen. For 11 of those participants, the quality of the MRI images was insufficient for quantification of abdominal visceral adipose tissue. MRI was performed in random subsample of those without contraindications. As a result, those who underwent the MRI have a slightly lower BMI (26.0 kg/m^2 versus 26.6 kg/m^2) and slightly less often a medical history of cardiovascular disease (4.1% versus 6.8%).

After exclusion of participants with a medical history of diabetes (n=161), extreme energy intake (<600 kcal/day or >5,000 kcal/day (n=19)), an incomplete FFQ (n=16) or missing data on smoking (n=2), education (n=22), ethnicity (n=3), energy-restricted diet (n=4) and physical activity (n=38), 2,304 participants were included in the analyses on visceral adipose tissue. Participants included in the analyses did not substantially differ from those excluded due to missing data regarding total body fat (30.7% for those without missing data versus 30.6% for those with missing data), visceral fat (87.6 cm2 versus 88.2 cm2), nor regarding dietary intake of the food groups. Liver fat was slightly lower in the participants with missing data (4.3% compared to 5.6%).

For the analyses with hepatic triglyceride content as an outcome, we excluded 424 participants without hepatic triglyceride content measurement. Due to the limited time available per subject it was not possible to check the spectra during the measurement and repeat the measurement when technical failures were present. As a consequence, ^{1}H-MRS of the liver could not be completed in 424 participants, for whom the majority were due to technical failures. However, the failure rate of the MR spectroscopy was not related to age (56 years for participants with hepatic triglyceride content measurement versus 55 years for participants without hepatic triglyceride content measurement), sex (47% male versus 46%), BMI (26.0 kg/m^2 versus 25.8 kg/m^2), visceral adipose tissue (90.1 cm^2 versus 87 cm^2), total body fat (30.7% versus 30.9%) or any of the food groups. Lastly, participants who drank more than 4 units of alcohol per day (n=165) were excluded for the analyses of hepatic triglyceride content.

The baseline characteristics of the study population participants are shown in **Table 1**. Whereas women had more total body fat, men had more visceral adipose tissue and hepatic triglyceride content.

Table 1. Baseline characteristics of of participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of abdominal fat depots and who were not using glucose-lowering therapy[1]

	Total population	Men (46.4%)	Women (53.6%)
Demographic variables			
Age (year)	55 (6)	56 (6)	55 (6)
Ethnicity (% white)	96	96	95
Education level (% high)[2]	47	51	43
Tobacco smoking (% current)	15	16	13
Menopausal state (% post)			58
Physical activity in leisure time (MET h/wk)	38.3 (33.1)	40.0 (38.8)	36.9 (28.1)
Dietary variables			
Dairy intake (g/d)	322 (196)	341 (223)	306 (172)
Meat intake (g/d)	83 (46)	96 (50)	72 (38)
Fish intake (g/d)	18 (17)	19 (19)	16 (15)
Fruit and vegetable intake (g/d)	326 (163)	304 (169)	345 (154)
Sweet snack intake (g/d)	82 (57)	89 (59)	75 (54)
Fat and oil intake (g/d)	35 (22)	41 (26)	29 (17)
Energy restricted diet (%)	10	6	14
Basal metabolic rate (MJ/d)	6.3 (1.1)	7.3 (0.7)	5.5 (0.6)
Body fat measures			
BMI (kg/m^2)	25.8 (3.9)	26.5 (3.5)	25.2 (4.0)
Total body fat (%)	30.7 (8.3)	24.5 (5.7)	36.1 (6.1)
Visceral adipose tissue (cm^2)	87.6 (54.2)	113.0 (58.7)	65.5 (39.8)
Hepatic triglyceride content (%)[3]	5.6 (7.7)	6.8 (8.2)	4.5 (7.2)
Fatty liver (HTGC>5.56%) (%)	28.2	37.5	20.2
Waist circumference (cm)	90.9 (12.6)	97.3 (10.4)	85.3 (11.3)
CVD risk factors			
CVD (%)	3.8	3.9	3.7
Lipid lowering medication (%)	7	11	4
Total serum cholesterol (mmol/L)	5.75 (1.04)	5.63 (1.04)	5.86 (1.03)
Fasting serum triglycerides (mmol/L)	1.23 (0.82)	1.43 (0.97)	1.06 (0.64)
HDL serum cholesterol (mmol/L)	1.58 (0.46)	1.35 (0.36)	1.79 (0.43)

[1]Values are means ± SDs. Results are based on analyses weighted toward the BMI distribution of the general population (n=2,304). BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoproteins; MET, metabolic equivalent of task.
[2]Low education: none, primary school, or lower vocational education as highest level of education.
[3]Mean HTGC only calculated in persons with HTGC measurement (n=1880)

Dietary intake of the main food groups in relation to visceral adipose tissue

We assessed the reproducibility of the dietary intake of the food groups in 100 participants who completed the FFQ twice with approximately three months in between. The individual measurement intraclass correlation coefficients of the food group dairy were 0.80, of fruit and vegetables 0.56, meat 0.83, sweet snacks 0.59, fish 0.64 and fats and oils 0.65. The individual intraclass correlation coefficient for total energy intake was 0.68.

In the total population, after adjustment for confounding factors, total body fat and a marker for an (un)healthy lifestyle, dietary intake of fruit and vegetables was associated with 1.2 cm^2 (95% CI -2.4, 0.0) less visceral adipose tissue (**Table 2**). Intake of plant-based fats and oils was also associated with 13.9 cm^2 less visceral adipose tissue (-23.7, -4.1). Dietary intake of dairy, fish, meat and sweet snacks was not associated with visceral adipose tissue (Table 2).

Tests for an interaction between the food groups and sex were nonsignificant, but we a priori decided to perform the analyses separately for men and women because of the large differences in body fat distribution. All associations were attenuated in the stratified analyses, although in women intake of dairy remained associated with visceral adipose tissue (-1.2 cm^2, -2.5, 0.0) (Table 2)

After a finer categorization of the food groups, yogurt seemed to drive the negative association between dairy and visceral adipose tissue in women (**Table 4**). Dietary intake of dairy, meat, and fruit and vegetables was more strongly associated with visceral adipose tissue in postmenopausal women than in premenopausal women (P-values for interactions: 0.56, 0.09 and 0.21) **(Supplemental table 1)**. The results remained similar after substituting dairy with other food groups (**Supplemental table 3**) and when including participants with diabetes (**Supplemental table 5**). The results did not differ when adjusting for potential under- or overreporting (data not shown).

Dietary intake of main food groups in relation to hepatic triglyceride content

In the total population of 1,715 participants with hepatic triglyceride content measurements, after adjustment for confounding factors, total body fat and a marker for an (un)healthy lifestyle, dietary intake of sweet snacks were associated with a 1.19-fold (95% CI 1.04, 1.37) higher hepatic triglyceride content (**Table 3**). The intake of dairy, fruit and vegetables, fish, meat, and fats and oils was not associated with hepatic triglyceride content (Table 3). In men and women separately, the associations were attenuated (Table 3).

Table 2. Difference in VAT (cm^2) with 95% confidence intervals per 100 g/day consumption of the food groups in of participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of abdominal fat depots and who were not using glucose-lowering therapy[1]

	Crude	**Multivariable**	**Multivariable + TBF**	**Multivariable + TBF + healthy diet**
Food groups	Difference in VAT (cm^2) with 95% CI	Difference in VAT (cm^2) with 95% CI	Difference in VAT (cm^2) with 95% CI	Difference in VAT (cm^2) with 95% CI
Dairy				
Total	0.1 (-1.3; 1.6)	-1.4 (-2.5; -0.3)*	-0.6 (-1.6; 0.4)	-0.6 (-1.6; 0.4)
Men	-0.6 (-2.6; 1.3)	-0.8 (-2.5; 0.9)	0.0 (-1.4; 1.5)	0.0 (-1.4; 1.5)
Women	-1.3 (-2.7; 0.2)	-2.0 (-3.4; -0.7)*	-1.3 (-2.5; 0.0)	-1.2 (-2.5; 0.0)
Meat				
Total	26.1 (20.2; 31.9)*	5.4 (0.1; 10.6)*	1.5 (-3.1; 6.0)	1.0 (-3.5; 5.6)
Men	10.9 (2.3; 19.4)*	3.9 (-4.3; 12.1)	-1.1 (-7.8; 5.6)	-1.4 (-8.0; 5.3)
Women	15.7 (8.7; 22.7)*	6.6 (0.7; 12.6)*	3.4 (-2.0; 8.7)	2.8 (-2.6; 8.2)
Fish				
Total	30.5 (13.4; 47.5)*	6.1 (-5.8; 18.1)	3.7 (-6.8; 14.2)	6.2 (-4.7; 17.1)
Men	14.6 (-8.3; 37.5)	5.2 (-14.3; 24.8)	1.9 (-15.2; 18.9)	3.0 (-14.6; 20.6)
Women	25.4 (6.8; 44.0)*	6.7 (-6.1; 19.5)	5.4 (-5.7; 16.6)	9.1 (-2.6; 20.9)
Fruit and vegetables				
Total	-3.1 (-4.8; -1.4)	-1.7 (-3.0; -0.4)*	-1.2 (-2.4; -0.0)*	-1.2 (-2.4; 0.0)
Men	-2.3 (-4.8; 0.2)	-1.8 (-4.2; 0.5)	-1.0 (-3.2; 1.2)	-0.8 (-3.1; 1.4)
Women	-0.4 (-2.1; 1.4)	-1.6 (-2.9; -0.2)*	-1.1 (-2.3; 0.1)	-1.2 (-2.4; 0.1)
Sweet snacks				
Total	4.3 (-0.7; 9.2)	-0.3 (-4.7; 4.2)	0.9 (-3.1; 5.0)	0.2 (-3.9; 4.4)
Men	-1.6 (-9.1; 5.8)	-0.6 (-8.1; 7.0)	4.0 (-2.3; 10.3)	3.7 (-2.6; 10.0)
Women	-0.0 (-5.0; 5.0)	0.4 (-4.8; 5.6)	0.6 (-4.5; 5.6)	-0.4 (-5.7; 5.0)
Fat and oils				
Total	33.5 (21.2; 45.9)	-8.2 (-20.2; 3.8)	-12.5 (-22.2; -2.7)*	-13.9 (-23.7; -4.1)*
Men	3.7 (-12.5; 19.8)	-13.1 (-30.9; 4.6)	-18.3 (-31.3; -5.3)*	-19.8 (-33.1; -6.4)*
Women	6.8 (-9.0; 22.6)	-1.2 (-14.4; 11.9)	-7.3 (-19.9; 5.3)	-8.3 (-20.9; 4.3)

[1]Multivariable: adjusted for age, total energy intake, smoking, education, ethnicity, physical activity in leisure time, basal metabolic rate, menopause and energy restricted diet. Results are based on analysis weighted toward the body mass index distribution of the general population (n=2,304, 1191 men and 1113 women). CI, confidence interval; TBF, total body fat; VAT, visceral adipose tissue.

*p-value<0.05

After the finer categorization, vegetables were more strongly associated with hepatic triglyceride content than fruit, and yogurt was most strongly associated with liver fat of all the dairy components (**Table 5**). The association between sweet snacks and hepatic triglyceride content was stronger in premenopausal women than in postmenopausal women (P-value for interaction 0.59) **(Supplemental table 2)**.

Substituting dairy with other food groups showed similar results as the multivariable analyses (**Supplemental table 4**), as did the analyses including participants with diabetes (**Supplemental table 6**). The results did not differ when adjusting for potential under- or overreporting (data not shown).

Table 3. Relative difference in HTGC with 95% confidence intervals per 100 g/day consumption of the food groups in of participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of abdominal fat depots and who were not using glucose-lowering therapy[1]

	Crude	Multivariable	Multivariable + TBF	Multivariable + TBF + healthy diet
Food groups	Relative difference in HTGC with 95% CI	Relative difference in HTGC with 95% CI	Relative difference in HTGC with 95% CI	Relative difference in HTGC with 95% CI
Dairy				
Total	0.98 (0.95; 1.02)	0.97 (0.93; 1.00)*	0.97 (0.94; 1.01)	0.97 (0.94; 1.01)
Men	0.98 (0.94; 1.03)	0.98 (0.94; 1.03)	0.99 (0.94; 1.03)	0.99 (0.94; 1.03)
Women	0.96 (0.91; 1.02)	0.95 (0.90; 0.99)*	0.96 (0.92; 1.01)	0.96 (0.92; 1.01)
Meat				
Total	1.48 (1.28; 1.71)*	1.14 (0.98; 1.33)	1.06 (0.92; 1.22)	1.05 (0.91; 1.20)
Men	1.16 (0.95; 1.41)	1.06 (0.86; 1.29)	0.97 (0.83; 1.15)	0.97 (0.82; 1.14)
Women	1.45 (1.16; 1.80)*	1.21 (0.97; 1.52)	1.10 (0.89; 1.36)	1.07 (0.87; 1.32)
Fish				
Total	1.18 (0.78; 1.80)	0.82 (0.57; 1.19)	0.79 (0.58; 1.08)	0.85 (0.61; 1.16)
Men	1.03 (0.63; 1.70)	0.96 (0.57; 1.63)	0.93 (0.59; 1.47)	0.96 (0.61; 1.50)
Women	1.08 (0.61; 1.90)	0.71 (0.45; 1.14)	0.69 (0.46; 1.04)	0.76 (0.48; 1.19)
Fruit and vegetables				
Total	0.95 (0.91; 0.99)	0.96 (0.92; 0.99)*	0.97 (0.93; 1.00)*	0.97 (0.94; 1.01)
Men	0.95 (0.90; 1.01)	0.97 (0.92; 1.02)	0.98 (0.94; 1.02)	0.99 (0.94; 1.03)
Women	0.98 (0.93; 1.03)	0.95 (0.91; 1.00)	0.96 (0.92; 1.00)	0.97 (0.93; 1.02)
Sweet snacks				
Total	1.17 (1.03; 1.33)*	1.22 (1.05; 1.42)*	1.21 (1.06; 1.39)*	1.19 (1.04; 1.37)*
Men	1.06 (0.91; 1.22)	1.13 (0.94; 1.35)	1.17 (1.01; 1.35)*	1.16 (0.99; 1.35)
Women	1.17 (0.95; 1.44)	1.29 (1.03; 1.63)*	1.26 (1.01; 1.57)*	1.23 (0.97; 1.54)
Fats and oils				
Total	1.84 (0.39; 2.44)	1.20 (0.88; 1.64)	1.16 (0.88; 1.53)	1.12 (0.85; 1.48)
Men	1.30 (0.91; 1.86)	1.26 (0.84; 1.88)	1.22 (0.86; 1.74)	1.20 (0.84; 1.71)
Women	1.42 (0.87; 2.30)	1.21 (0.75; 1.94)	1.08 (0.68; 1.70)	1.04 (0.66; 1.65)

[1]Multivariable: adjusted for age, total energy intake, smoking, education, ethnicity, physical activity in leisure time, basal metabolic rate, menopause, alcohol consumption and energy restricted diet. Results are based on analysis weighted toward the body mass index distribution of the general population (n=1,715, 831 men and 884 women). CI, confidence interval; HTGC, hepatic triglyceride content; TBF, total body fat.
*p-value<0.05

Table 4. Difference in VAT (cm²) with 95% confidence intervals per 100 g/day consumption of the food groups in of participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of abdominal fat depots and who were not using glucose-lowering therapy[1]

	Crude	Multivariable	Multivariable + TBF	Multivariable + TBF + healthy diet
Food groups	Difference in VAT (cm²) with 95% CI	Difference in VAT (cm²) with 95% CI	Difference in VAT (cm²) with 95% CI	Difference in VAT (cm²) with 95% CI
Cheese				
Total	6.9 (-4.2; 17.9)	-0.2 (-8.7; 8.3)	-0.1 (-7.7; 7.6)	-0.2 (-7.9; 7.5)
Men	16.8 (2.2; 31.4)*	7.8 (-5.8; 21.4)	0.4 (-10.7;11.6)	0.2 (-10.9; 11.4)
Women	-6.6 (-19.5; 6.3)	-8.6 (-19.0; 1.9)	-4.2 (-13.8; 5.4)	-4.3 (-13.9; 5.3)
Milk				
Total	1.5 (-0.2; 3.2)	-0.3 (-1.5; 0.8)	0.2 (-0.9; 1.2)	0.1 (-0.9; 1.2)
Men	0.19 (-2.1; 2.5)	-0.1 (-2.0; 1.8)	0.7 (-0.9; 2.4)	0.7 (-1.0; 2.3)
Women	-0.4 (-1.9; 1.1)	-0.6 (-1.7; 0.5)	-0.2 (-1.4;0.9)	-0.3 (-1.4; 0.8)
Yogurt				
Total	-8.8 (-13.1; -4.5)*	-7.4 (-11.0; -3.9)*	-5.2 (-7.8; -2.6)*	-5.0 (-7.6; -2.4)*
Men	-7.4 (-12.6; -2.2)*	-6.0 (-11.3; -0.6)*	-3.9 (-7.8; 0.0)	-3.7 (-7.6; 0.2)
Women	-5.9 (-10.0; -1.7)*	-9.2 (-12.3; -6.1)*	-6.6 (-9.4; -3.7)*	-6.3 (-9.1; -3.4)*
Cream butter				
Total	-3.5 (-48.0; 40.9)	-9.6 (-37.8; 18.5)	-6.8 (-33.8; 20.2)	-7.3 (-34.6; 20.0)
Men	-29.8 (-85.7; 26.1)	-43.8 (-97.2; 9.6)	-30.3 (-69.7; 9.0)	-27.8 (-67.0; 11.4)
Women	-7.4 (-52.4; 37.5)	13.0 (-23.5; 49.5)	10.4 (-27.3; 48.1)	10.8 (-27.0; 48.7)
Fruit				
Total	-5.2 (-7.7; -2.7)	-2.3 (-4.2; -0.4)	-1.2 (-2.9; 0.5)	-1.2 (-2.9; 0.5)
Men	-3.9 (-7.3; -0.4)*	-2.6 (-5.9; 0.8)	-0.6 (-3.5; 2.3)	-0.5 (-3.4; 2.3)
Women	-1.7 (-4.1; 0.8)	-1.9 (-3.9; 0.0)	-1.3 (-3.1; 0.5)	-1.3 (-3.1; 0.5)
Vegetables				
Total	-0.8 (-3.6; 2.1)	-1.2 (-3.3; 1.0)	-1.5 (-3.4; 0.4)	-1.5 (-3.4; 0.4)
Men	-0.4 (-5.0; 4.2)	-0.8 (-5.0; 3.5)	-2.1 (-5.9; 1.6)	-1.8 (-5.6; 1.9)
Women	1.5 (-1.3; 4.3)	-1.5 (-3.7; 0.7)	-1.3 (-3.2; 0.7)	-1.3 (-3.3; 0.8)
Cake				
Total	4.7 (-4.9; 14.3)	-1.8 (-9.9; 6.3)	-3.3 (-10.1; 3.5)	-3.8 (-10.6; 3.1)
Men	-2.1 (-16.5; 12.2)	0.1 (-13.7; 13.9)	-0.1 (-10.6; 10.5)	0.1 (-10.5; 10.7)
Women	3.6 (-6.8; 14.1)	-1.6 (-10.4; 7.2)	-2.2 (-10.3; 5.9)	-3.3 (-11.5; 4.8)
Candy				
Total	1.5 (-6.2; 9.2)	0.1 (-6.2; 6.5)	1.7 (-4.0; 7.4)	1.0 (-4.9; 6.9)
Men	-1.4 (-12.6; 9.9)	-2.4 (-14.1; 9.2)	1.9 (-7.6; 11.3)	1.6 (-7.9; 11.1)
Women	-0.4 (-7.2; 6.3)	2.4 (-4.2; 9.0)	2.7 (-3.8; 9.3)	1.8 (-4.9; 8.6)
Margarine				
Total	34.1 (20.0; 48.2)	-10.5 (-23.0; 2.0)	-14.2 (-24.3; -4.1)*	-16.6 (-26.8; -6.4)*
Men	-2.7 (-21.0; 15.6)	-18.3 (-36.3;-0.4)*	-19.2 (-32.6; -5.8)*	-21.1 (-34.9; -7.3)*
Women	11.1 (-7.1; 29.3)	2.1 (-11.7; 15.8)	-7.0 (-20.1; 6.0)	-9.4 (-22.5; 3.8)
Oils				
Total	27.1 (-4.3; 58.5)	10.0 (-15.4; 35.4)	2.1 (-20.1; 24.3)	5.6 (-17.2; 28.3)
Men	24.0 (-14.9; 63.0)	13.2 (-23.9;50.4)	-10.4 (-41.6; 20.9)	-8.2 (-40.0; 23.7)
Women	4.1 (-35.1; 43.4)	5.4 (-27.6; 38.4)	9.8 (-20.3; 39.9)	13.8 (-16.5; 44.2)

[1]Multivariable: adjusted for age, total energy intake, smoking, education, ethnicity, physical activity in leisure time, basal metabolic rate, menopause and energy restricted diet. Results are based on analysis weighted toward the body mass index distribution of the general population (n=2304, 1191 men and 1113 women). CI, confidence interval; TBF, total body fat; VAT, visceral adipose tissue.
*p-value<0.05

Table 5. Relative difference in HTGC with 95% confidence intervals per 100 g/day consumption of the food groups groups in of participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of abdominal fat depots and who were not using glucose-lowering therapy[1]

	Crude	Multivariable	Multivariable + TBF	Multivariable + TBF + healthy diet
Food groups	Relative difference in HTGC with 95%CI	Relative difference in HTGC with 95%CI	Relative difference in HTGC with 95%CI	Relative difference in HTGC with 95%CI
Cheese				
Total	1.11 (0.85; 1.46)	1.01 (0.78; 1.32)	1.01 (0.80; 1.29)	1.00 (0.79; 1.28)
Men	1.34 (0.94; 1.89)	1.34 (0.96; 1.86)	1.10 (0.82; 1.48)	1.09 (0.81; 1.47)
Women	0.94 (0.64; 1.38)	0.82 (0.56; 1.20)	0.98 (0.67; 1.44)	0.97 (0.67; 1.43)
Milk				
Total	1.00 (0.95; 1.05)	0.97 (0.94; 1.01)	0.98 (0.95; 1.01)	0.98 (0.95; 1.01)
Men	0.99 (0.93; 1.05)	0.98 (0.93; 1.04)	0.99 (0.94; 1.04)	0.99 (0.94; 1.04)
Women	0.98 (0.92; 1.04)	0.97 (0.92; 1.02)	0.98 (0.93; 1.02)	0.97 (0.93; 1.02)
Yogurt				
Total	0.89 (0.81; 0.97)	0.89 (0.82; 0.98)*	0.91 (0.85; 0.99)	0.92 (0.85; 1.00)*
Men	0.91 (0.84; 0.99)*	0.94 (0.85; 1.04)	0.95 (0.87; 1.03)	0.95 (0.88; 1.03)
Women	0.88 (0.77; 1.02)	0.82 (0.72; 0.94)*	0.87 (0.76; 0.99)*	0.88 (0.78; 1.01)
Cream butter				
Total	0.58 (0.25; 1.34)	0.75 (0.38; 1.46)	0.79 (0.43; 1.47)	0.79 (0.43; 1.46)
Men	0.57 (0.13; 2.41)	0.90 (0.20; 4.11)	1.07 (0.26; 4.41)	1.09 (0.23; 5.08)
Women	0.50 (0.20; 1.22)	0.74 (0.34; 1.59)	0.72 (0.37; 1.39)	0.69 (0.35; 1.35)
Fruit				
Total	0.94 (0.89; 0.99)*	0.96 (0.92; 1.01)	0.98 (0.94; 1.03)	0.98 (0.94; 1.03)
Men	0.94 (0.89; 1.00)	0.96 (0.90; 1.02)	0.98 (0.93; 1.04)	0.99 (0.93; 1.04)
Women	0.98 (0.91; 1.06)	0.96 (0.89; 1.03)	0.98 (0.90; 1.05)	0.98 (0.91; 1.06)
Vegetables				
Total	0.94 (0.88; 1.01)	0.94 (0.88; 1.00)*	0.93 (0.88; 0.98)	0.94 (0.89; 1.00)*
Men	0.96 (0.85; 1.07)	0.98 (0.89; 1.07)	0.97 (0.89; 1.05)	0.98 (0.90; 1.07)
Women	0.96 (0.89; 1.05)	0.93 (0.85; 1.00)	0.92 (0.86; 0.99)*	0.93 (0.86; 1.00)
Cake				
Total	1.28 (1.00; 1.62)*	1.21 (0.96; 1.51)	1.11 (0.91; 1.37)	1.10 (0.89; 1.35)
Men	1.05 (0.78; 1.42)	1.09 (0.78; 1.53)	1.00 (0.76; 1.32)	1.00 (0.76; 1.33)
Women	1.38 (0.97; 1.96)	1.24 (0.91; 1.69)	1.19 (0.88; 1.60)	1.15 (0.85; 1.55)
Candy				
Total	1.18 (0.97; 1.43)	1.20 (0.98; 1.47)	1.23 (1.03; 1.46)*	1.20 (1.00; 1.44)
Men	1.12 (0.92; 1.37)	1.11 (0.88; 1.39)	1.20 (1.00; 1.45)*	1.19 (0.99; 1.44)
Women	1.21 (0.88; 1.67)	1.26 (0.93; 1.71)	1.21 (0.91; 1.61)	1.18 (0.87; 1.58)
Margarine				
Total	2.02 (1.47; 2.79)*	1.19 (0.85; 1.68)	1.15 (0.86; 1.53)	1.09 (0.81; 1.47)
Men	1.28 (0.84; 1.96)	1.15 (0.72; 1.82)	1.18 (0.80; 1.72)	1.14 (0.77; 1.69)
Women	1.64 (0.99; 2.71)	1.22 (0.75; 1.98)	1.03 (0.66; 1.61)	0.97 (0.61; 1.52)
Oils				
Total	1.18 (0.54; 2.53)	1.03 (0.49; 2.17)	0.88 (0.47; 1.65)	0.96 (0.51; 1.82)
Men	1.20 (0.46; 3.08)	1.26 (0.47; 3.41)	0.88 (0.39; 2.02)	0.92 (0.40; 2.11)
Women	0.98 (0.32; 3.01)	1.06 (0.37; 3.07)	1.10 (0.40; 2.99)	1.29 (0.47; 3.56)

[1]Multivariable: adjusted for age, total energy intake, smoking, education, ethnicity, physical activity in leisure time, basal metabolic rate, menopause, alcohol consumption and energy restricted diet. Results are based on analysis weighted toward the body mass index distribution of the general population (n=1,715, 831 men and 884 women). CI, confidence interval; HTGC, hepatic triglyceride content; TBF, total body fat.
*p-value>0.05

DISCUSSION

In this population-based study of participants aged 40 to 65 without contraindications for a MRI, we examined for the first time to what extent dietary intake of the main food groups was specifically associated with visceral fat and liver fat content, as assessed with MRI and ^{1}H-MRS. In the total population, dietary intake of fruit and vegetables and plant-based fats and oils was associated with less visceral fat, and intake of sweet snacks was associated with more liver fat. Although confidence intervals were wide, both in the total population and in men and women separately, a similar pattern of positive associations with intake of sweet snacks and inverse associations with intake of fats and oils, dairy, and fruit and vegetables could be observed.

The observed associations were largely explained by total body fat. On the one hand, the remaining observed associations may suffer from residual confounding due to imperfectly measured total body fat. On the other hand, the results of the associations of fruit and vegetables and plant-based fats and oils with visceral fat and that of sweet snacks with liver fat that remained in the total population after multivariate adjustment including total body fat and a marker for an (un)healthy diet, support the presence of specific associations of certain food groups with visceral and liver fat, and need to be confirmed in larger studies.

Although few studies have investigated the association between food groups and visceral adipose tissue and hepatic triglyceride content, our findings are in accordance with the current literature on food groups in relation to cardiometabolic diseases and the current food group-based dietary guidelines in the European region [25] and they support the dietary patterns of the DASH diet and Alternative Healthy Eating Index (AHEI) [43]. Dietary intake of meat and sugar-sweetened beverages has been associated with an increased risk of type 2 diabetes [28, 44] and intake of dairy and fruits with a lower risk of type 2 diabetes [28, 45]. In a recent meta-analysis, dietary intake of fish and fruit and vegetables has also been associated with a decreased risk of all-cause mortality, whereas red meat and processed meat were associated with an increased risk [46].

Dairy was negatively associated with visceral fat in women, and this association was mostly driven by yogurt, which supports previous results from the Women's Health Initiative Observational Study showing that high yogurt consumption significantly decreased diabetes risk [47]. When butter was excluded from the dairy food group, the associations remained similar, indicating that butter intake did not contribute to the inverse association.

In our study, we did not observe an association between fish intake and liver fat or visceral fat. Although the point estimate for fish intake and visceral adipose tissue was positive, confidence intervals were very wide. It must be noted that we could not distinguish between fresh fish and fried fish on the basis of our food frequency questionnaire, and thus, this food group was relatively heterogeneous. However, a recent meta-analysis showed that fish intake was inversely associated with diabetes in Asian populations but positively associated with diabetes in Western populations, in which no distinction was made between fresh and fried fish [48].

In this study, meat was not associated with visceral or liver fat. However, our food frequency questionnaire did not make a distinction between poultry, red meat or processed meat. Associations with red meat and processed meat might potentially be stronger than those observed with total meat. Even though the exact mechanism remains unidentified, the dietary cholesterol, protein, heme-iron, advanced glycation products or preservatives such as sodium and nitrites/nitrates in meat have been hypothesized to be responsible for the positive association with visceral adipose tissue and diabetes [49]. Regarding dairy, calcium, vitamin D, magnesium, fatty acids, protein and the effect on satiety are hypothesized to underlie the beneficial effect [45]. However, dairy products are often differentially categorized across different studies [23], making it difficult to compare. Different dairy products, such as fermented dairy or low- and high-fat dairy, might be associated differently with cardiometabolic outcomes, but all are categorized as dairy. Other nutrients and dietary aspects have already been shown to be associated with measures of adiposity, such as dietary fiber with less visceral adipose tissue [50], high-glycemic index diets with higher waist circumference [51] and high protein (either animal or plant) and n-6 polyunsaturated fatty acids with less hepatic triglyceride content [52, 53]. However, food groups instead of single nutrients in relation to visceral adipose tissue and hepatic triglyceride content have not yet been studied.

Strengths of this study include the direct assessment of visceral fat and hepatic triglyceride content by MRI and ^{1}H-MRS, respectively, in a relatively large sample size. Additionally, the extensive phenotypic measurements allowed adjustment for many potential confounding factors, and the large study population enabled us to investigate possible sex differences. A limitation of this study is that the FFQ was self-administered and therefore prone to measurement error. When assessing reproducibility in a random subsample, the ICCs of fruit and vegetables and sweet snacks were moderate to low, which could be due to seasonal variation, but might also indicate potential over- or underreporting. Furthermore, a limitation of studying food groups may be that they cover a broad range of food products and might comprise both relatively healthy and unhealthy products. As we could not distinguish between white meat, red meat and processed meat, this might have attenuated our associations due to regression dilution. Moreover, the observational cross-sectional design of this study precludes any causal inference, and residual confounding, for example due to unmeasured or insufficiently measured lifestyle factors, may still be present despite our efforts to minimize confounding as much as possible. Additionally, potential selection bias might have occurred due to missing data. However, the number of participants excluded due to missing data was limited (n=96) and the failure rate of liver fat measurement was not dependent on sex, age or body fat measurements, so we do not expect this factor to substantially alter our results. Lastly, our study population consisted primarily of white, middle-aged participants, and there might be differences in dietary habits [54] and visceral adipose tissue [55] and hepatic triglyceride content accumulation [56] between different ethnic populations. Therefore, our findings need to be confirmed in prospective studies and in other ethnic groups.

In conclusion, in this population-based study in middle-aged men and women without contra-indications of an MRI, dietary intake of plant-based fats and oils and fruits and vegetables was associated with less visceral adipose tissue. Intake of sweet snacks was associated with more liver fat. Larger prospective studies on the relation between a food group and ectopic fat accumulation are needed to confirm whether associations between dietary intake of certain food groups are specifically associated with visceral fat or liver fat. In addition, intervention studies are needed to establish to what extent dietary changes can specifically reduce ectopic fat accumulation and the risk of cardiometabolic disease.

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4

Adherence to dietary guidelines in relation to visceral fat and liver fat in middle-aged men and women: the NEO study

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ABSTRACT

Background
It is unclear to what extent adherence to dietary guidelines may specifically affect visceral fat and liver fat. We aimed to study the association between the Dutch Healthy Diet Index (DHD-index) and total body fat, visceral adipose tissue (VAT) and hepatic triglyceride content (HTGC) in middle-aged men and women.

Design
In this cross-sectional study, VAT was assessed by magnetic resonance imaging (MRI) in 2 580 participants, and HTGC by proton-MR spectroscopy in 2 083 participants. Habitual dietary intake and physical activity were estimated by questionnaire. Adherence to the current Dutch dietary guidelines was estimated by the 2015 DHD-index score based on thirteen components (vegetables, fruit, wholegrain products, legumes, nuts, dairy, fish, tea, liquid fats, red meat, processed meat, sweetened beverages and alcohol). The DHD-index ranges between 0 and 130, with a higher score indicating a healthier diet. We used linear regression to examine associations of the DHD-index with VAT and HTGC, adjusted for age, smoking, education, ethnicity, basal metabolic rate, energy restricted diet, menopausal state, physical activity, total energy intake, and total body fat. We additionally excluded the components one by one to examine individual contributions to the associations.

Results
Included participants (43% men) had a mean (SD) age of 56 (6) years and DHD-index score of 71 (15). A 10-point higher DHD-index score was associated with 2.3 cm^2 less visceral fat (95% CI; -3.5; -1.0 cm^2) and less liver fat (0.94 times, 95% CI; 0.90; 0.98). Of all components, exclusion of dairy attenuated the associations with TBF and VAT.

Conclusions
Adherence to the dietary guidelines as estimated by the DHD-index was associated with less total body fat, and with less visceral and liver fat after adjustment for total body fat. These findings might contribute to better understanding of the mechanisms underlying associations between dietary habits and cardiometabolic diseases.

INTRODUCTION

The prevalence of obesity is increasing worldwide. In particular abdominal obesity is a well-established risk factor for the metabolic syndrome, diabetes mellitus and cardiovascular diseases [1, 2]. The excess risk of abdominal obesity is hypothesized to be due to the accumulation of fat in the visceral area and in and around the organs (ectopic fat) [2], such as in the liver. Visceral adipose tissue (VAT) and hepatic triglyceride content (HTGC) have been associated with insulin resistance, metabolic risk factors and cardiovascular disease [3-6]. Due to these multiple health-related consequences, visceral fat and liver fat are important targets for battling cardiometabolic diseases.

Together with physical activity, diet is an essential modifiable risk factor for obesity and obesity-related chronic diseases [7]. Recently, dietary guidelines have started to shift from nutrient-based to food-based and dietary patterns, as humans do not consume separate nutrients but rather combinations of foods [8-10]. Also, some nutrient effects might be too small to detect separately [11] and different nutrients might be strongly correlated or even interact with each other, making it hard to disentangle their effects. As nutrient intakes are often associated with certain dietary patterns, analyses including only one nutrient might therefore be confounded by dietary patterns [9].

Numerous dietary indices of adherence to a healthy diet have been developed over the last two decades, among which the (Alternative) Healthy Eating Index (HEI) [12], the Healthy Diet Indicator (HDI) [13] and the Diet Quality Index (DQI) [14]. The HDI has been associated with both all-cause and cardiovascular disease mortality and the DQI with circulatory disease mortality in women [15]. The HEI has also been associated with obesity [16], which might be an underlying mechanism for the association between the HEI and cardiovascular disease, as shown in previous research [15].

However, it remains unknown whether adherence to dietary guidelines has specific effects on visceral fat and hepatic triglyceride content or merely on overall body fat. Therefore, we aimed to study the association between the Dutch Healthy Diet Index (DHD-index) and total body fat (TBF), visceral adipose tissue and liver fat. In addition, we explored which components of the DHD-index contributed most to the associations with total body fat, visceral fat and liver fat.

METHODS

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study in 6 671 individuals aged 45 to 65 years, with an oversampling of persons with a BMI of 27 kg/m^2 or higher. Detailed information about the study design and data collection has been described elsewhere [17]. Men and women aged between 45 and 65 years with a self-reported BMI of 27 kg/m^2 or higher living in the greater area of Leiden (in the West of The Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI, allowing for a reference distribution of BMI.

Participants visited the NEO study center of the Leiden University Medical Center after an overnight fast. Prior to the NEO study visit, participants completed a questionnaire about demographic, lifestyle, and clinical information, in addition to a food frequency questionnaire. At the study center, the participants completed a screening form, asking about anything that might create a health risk or interfere with magnetic resonance imaging (MRI) (most notably metallic devices, claustrophobia, or a body circumference of more than 1.70 m). Of the participants who were eligible for MRI, approximately 35% were randomly selected to undergo direct assessment of abdominal fat.

The present study is a cross-sectional analysis of the baseline measurements. We excluded participants with implausibly low or high total energy intake (<600 kcal or >5 000 kcal/day), which are somewhat less conservative cut-off points for high energy intake than other cohort studies [12] because of our smaller sample size. Moreover, we excluded participants with missing data on dietary intake or potential confounding factors. For the analyses on liver fat, we additionally excluded participants who consumed more than four standard units of alcohol per day.

The study was approved by the medical ethics committee of the Leiden University Medical Center and conducted according to the declaration of Helsinki. All participants gave written informed consent.

Data collection

On the questionnaire, participants reported ethnicity by self-identification in eight categories which we grouped into white (reference) and other. Tobacco smoking was reported in the three categories current, former, and never smoking (reference). Highest level of education was reported in 10 categories according to the Dutch education system and grouped into high (including higher vocational school, university, and post-graduate education) versus low education (reference). Participants reported their medical history of diabetes and cardiovascular diseases. Pre-existing cardiovascular disease was defined as myocardial infarction, angina, congestive heart failure, stroke, or peripheral vascular disease. Body weight was measured without shoes and one kilogram was subtracted from the body weight. Percent body fat was estimated using bioelectrical impedance analysis (BIA) with the Tanita foot-to-foot (FF) BIA system TBF-300A Body Composition Analyzer [18]. BMI was calculated by dividing the weight in kilograms by the height in meters squared. Menopausal state was categorized in pre-, and postmenopausal state according to information on ovariectomy, hysterectomy and self-reported state of menopause in the questionnaire. Basal metabolic rate was calculated based on age, sex, height and weight according to the Mifflin-St Jeor equation [19]. Participants reported the frequency, duration and intensity of their habitual physical activity during leisure time using the Short QUestionnaire to ASsess Health-enhancing physical activity, which was expressed in hours per week of metabolic equivalents (MET-h/week) [20].

Dutch Healthy Diet Index

Habitual dietary intake of all participants was estimated using a semi-quantitative self-administered 125-item food frequency questionnaire (FFQ) [21, 22]. In this questionnaire, participants reported their frequency of intake of foods during the past month (times per day, week, month, never). This was combined with the assessment of serving size (spoons of potatoes, pieces of fruit, etc). Dietary intake of nutrients and total energy was estimated using the Dutch Food Composition Table (NEVO-2011).

Based on the FFQ, we calculated the DHD-index for each participant, which is a continuous score and represents the adherence to the Dutch Guidelines for Healthy Diet of 2015 as described by the Health Council of the Netherlands and originally consists of fifteen components [23]. Every index component has a maximum of 10 points, depending on the cut-off as described by the guidelines for each component: vegetables (≥200 grams per day), fruit (≥200 grams per day), wholegrain products (ratio of whole grains to refined grains ≥1), legumes (≥10 grams per day), unsalted nuts (≥ 15 grams per day), dairy (between 300 and 450 grams per day), fish (≥ 15 grams per day), tea (≥450 grams per day), replacing hard fats by liquid fats (ratio of liquid to solid fats ≥13), coffee (consumption of only filtered coffee), red meat (≤ 45 grams per day), processed meat (0 grams per day), sweetened beverages (0 grams per day), alcohol (≤10 grams per day) and salt (≤1.9 grams of sodium per day). Assessing the adherence to these guidelines is based on five types of components in the Dutch Healthy Diet Index: 1) adequacy components (minimum consumption recommended, e.g. vegetables, fruit, wholegrain products, legumes and

nuts), 2) moderation components (limited consumption recommended, e.g. red meat, processed meat, sweetened beverages, alcohol and salt), 3) optimum components (consumption between certain limits recommended, e.g. dairy), 4) qualitative components (recommended consumption depending on quality of product, e.g. coffee), and 5) ratio components (a certain ratio of consumption is recommended, e.g. fats and oils and wholegrain products) [23]. As a result, the total score can range between 0 and 150. A higher score means a better adherence to the 2015 Dutch Guidelines for a Healthy Diet. For the present study, we used an adapted version of the DHD-index with thirteen components instead of the original fifteen because we were not able to estimate the two components consumption of unfiltered coffee, and of sodium on the basis of the FFQ used in our study. As a result, the DHD-index in our study ranges between 0 and 130.

Visceral fat area and hepatic triglyceride content by imaging techniques

Imaging was performed on a 1.5 Tesla MR system (Philips Medical Systems, Best, the Netherlands). Visceral adipose tissue (VAT) was quantified by a turbo spin echo imaging protocol using MRI. At the level of the fifth lumbar vertebra, three transverse images each with a slice thickness of 10 mm were obtained during a breath-hold. Visceral fat area was converted from the number of pixels to centimeters squared using in-house-developed software (MASS, Medis, Leiden, the Netherlands) and the average of three slices was used in the analyses [17].

Hepatic triglyceride content was quantified by proton-MR spectroscopy (^{1}H-MRS) of the liver [24]. An 8 ml voxel positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Sixty-four averages were collected with water suppression. Spectra were obtained with an echo time of 26 ms and a repetition time of 3,000 ms. Data points (1,024) were collected using a 1,000 Hz spectral line. Without changing any parameters, spectra without water suppression, with a repetition time of 10 s, and with four averages were obtained as an internal reference. ^{1}H-MRS data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium), as described previously [25]. Hepatic triglyceride content relative to water was calculated the sum of signal amplitudes of methyl and methylene divided by the signal amplitude of water and then multiplied by 100.

Statistical analyses

In the NEO study there is an oversampling of persons with a BMI of 27 kg/m^2 or higher. To correctly represent associations in the general population [26], adjustments for the oversampling of individuals with a BMI ≥ 27 kg/m^2 were made. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality [27], whose BMI distribution was similar to the BMI distribution of the general Dutch population [28]. All results were based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI ≥ 27 kg/m^2. As a result of the weighted analyses, percentages and proportions are given instead of numbers of participants. Other baseline characteristics are expressed as mean with standard deviation.

We performed linear regression analyses with multiple models. First, we studied the association between the DHD-index (per 10 points) with overall adiposity, as measured by total body fat (%). We performed both a crude model, and a multivariable model adjusted for age, sex, smoking, education, ethnicity, basal metabolic rate, menopausal state, physical activity, adherence to an energy restricted diet and total energy intake. After this, we examined the associations between the DHD-index with visceral fat and liver fat content. To examine whether the associations were specific for visceral fat and liver fat instead of merely representing effects on total adiposity, we additionally corrected for total body fat in a separate model. For this, we calculated variance inflation factors (VIFs) to check for multicollinearity in our models between visceral fat or liver fat and total body fat. The VIF values were below 10 in all models and were considered appropriate. Correlation coefficients between total body fat and visceral fat (0.13) and total body fat and liver fat (0.17) were also low, indicating that additional adjustment for total body fat does not lead to multicollinearity.

Lastly, to examine which component most strongly contributed to the associations of the DHD-index with visceral fat or liver fat, we performed analyses in which we subsequently left out one component at the time and additionally adjusted for that component. We reasoned that a component has an important contribution to the association if the association attenuates towards the null after leaving that component out. Linearity of the DHD-index and its components with visceral fat and liver fat was checked by adding a quadratic term to the main multivariable model and visual inspection of scatter plots.

We performed several subgroup analyses. Because total body fat and visceral adipose tissue and hepatic triglyceride content may differ greatly between persons with and without obesity [29], between men and women, and between pre- and postmenopausal women [30], we stratified the multivariable model not including total body fat by these variables. We additionally stratified the multivariable models for liver fat by the rs738409 single nucleotide polymorphism in the patatin-like phospholipase domain containing 3 (PNPLA3) gene, because carriers might have, in part, genetically induced liver fat which might be less strongly related to dietary habits [31].

Due to a skewed distribution of HTGC, we used the natural logarithm of this variable in the analyses. For interpretation of the results, we back transformed the regression coefficients of HTGC towards a ratio with 95% confidence interval, which is associated with a 10 points higher DHD-index. Such ratio, for example a ratio of 1.2, can be interpreted as each five points higher DHD-index being associated with a 1.2-fold increased HTGC, which would reflect an increase in liver fat from, for example, 5% to 6%. Regression coefficients of total body fat represent an absolute difference in TBF in % per 10 points higher DHD-index, and those of VAT an absolute difference in VAT in cm^2.

As participants with diabetes might have altered their diet as a result of the diagnosis, we repeated all analyses excluding participants with a medical history of diabetes mellitus. We performed all analyses using STATA statistical Software (Statacorp, College Station, Texas, USA), version 14.

RESULTS

A total of 6 671 participants were included in the NEO study between September 2008 and October 2012. For the analyses with total body fat as an outcome, we excluded participants without a body fat assessment (n=31), implausible energy intake (n=62) or missing energy intake (n=4), an incomplete food frequency questionnaire (n=23) or missing data on smoking (n=7), education (n=62), ethnicity (n=8) or physical activity (n=114), leaving a total of 6,630 participants.

For the analyses on VAT, we additionally excluded participants without an MRI of the abdomen (n=3 912), which was performed in a random subsample of participants without contraindications. As a result, those who underwent the MRI have a slightly lower BMI (25.9 kg/m^2 versus 26.6 kg/m^2) and slightly less often a medical history of cardiovascular disease (4.1% versus 6.6%) than those without MRI. All other characteristics were similar. The total study population for the analyses on VAT contained 2 450 participants.

For the analyses with HTGC as an outcome, we additionally excluded participants without HTGC measurement (n=464). The majority of this missing values was due to technical failure, as it was not possible to check the spectra and repeat the measurement in the limited time available per participant. However, the failure rate of the MR spectroscopy was not related to age (55 years for participants with hepatic triglyceride content measurement versus 56 years for participants without hepatic triglyceride content measurement), sex (47% men versus 48%), BMI (25.9 kg/m^2 versus 26.2 kg/m^2), visceral adipose tissue (89 cm^2 versus 94 cm^2) or the DHD-index (59.5 versus 58.8 points). Lastly, we excluded participants who consumed 40 grams of alcohol or more (4 standard glasses) per day (n=176) and one participant for whom the natural logarithm of HTGC could not be calculated, leaving a total of 1 809 participants.

The baseline characteristics of the total population for the analyses on total body fat stratified by tertiles of the DHD-index are shown in **Table 1**. Participants in the highest tertile and thus who adhere the most to the dietary guidelines, more often had a high education, were female, and non-smoking.

Dutch Healthy Diet Index in relation to total body fat

After adjustment for potential confounding factors, 10 points higher on the DHD-index was associated with 0.2 % less total body fat (95% CI -0.3; -0.1 %) (**Table 2**). Of all components, leaving out the processed meat component attenuated the association, as did dairy and fruit (**Figure 1**). Results were similar in men and women (data not shown).

After stratification by BMI, results were similar for both groups **(Supplemental table 1)**. The association between the DHD-index and total body fat was somewhat stronger in postmenopausal women than in premenopausal women (Supplemental table 1).

Dutch Healthy Diet Index in relation to visceral adipose tissue

After adjustment for potential confounding factors and total body fat, the DHD-index was inversely associated with visceral adipose tissue (-2.2 cm^2 per 10-points higher on the DHD-index, 95% CI -3.5; -1.0 cm^2) (**Table 2**). Of all components, leaving out the dairy component of the DHD-index slightly attenuated the association (**Figure 2**). Results were similar in men and women (data not shown).

When stratified by BMI, the association between the DHD-index and VAT was similar in participants with or without obesity and in post- and premenopausal women **(Supplemental table 1)**.

Table 1. Baseline characteristics stratified by tertiles of the DHD-index of participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age

	DHD-index		
	Tertile 1 (19.6-64.3)	**Tertile 2 (≥64.3-77.8)**	**Tertile 3 (≥77.8-119.1)**
Demographic variables			
Age (year)	55 (7)	56 (6)	56 (5)
Sex (% men)	55.6	43.7	31.0
Ethnicity (% white)	94.5	95.4	94.9
Education level (% high)[1]	37.1	46.2	54.8
Tobacco smoking (% current)	28.8	13.1	6.6
Menopausal state (% post in women)[2]	56.6	57.4	65.4
Physical activity in leisure time (MET h/wk)	26.3 [12.0-44.3]	30.0 [16.9-50.5]	32.7 [18.5-52.3]
Dietary variables			
DHD-index	54.4 (7.8)	71.0 (4.0)	87.3 (6.7)
Fruit and vegetable intake (g/d)	235 (142)	322 (154)	419 (142)
Alcohol intake (g/d)	18.2 [4.0-31.3]	9.2 [2.6-20.8]	7.5 [2.0-14.3]
Energy restricted diet (%)	9.4	11.6	14.5
Basal metabolic rate (MJ/d)	6.6 (1.2)	6.4 (1.1)	6.0 (0.9)
Body fat measures			
BMI (kg/m^2)	26.9 (4.8)	26.5 (4.5)	25.5 (3.9)
Total body fat (%)			
Men	25.7 (6.6)	25.1 (6.0)	23.7 (5.3)
Women	37.4 (7.3)	37.3 (6.6)	36.3 (5.7)
Visceral adipose tissue (cm^2)[3]			
Men	123.7 (67.4)	114.6 (59.1)	104.0 (51.4)
Women	69.7 (44.2)	68.3 (46.2)	63.3 (34.2)
Hepatic triglyceride content (%)[4]			
Men	4.9 [2.0-13.1]	3.7 [2.2-7.8]	3.2 [1.9-6.2]
Women	2.2 [1.2-5.8]	1.8 [1.1-5.1]	1.6 [1.1-3.3]
Waist circumference (cm)			
Men	99.7 (12.2)	98.5 (11.2)	96.0 (9.7)
Women	88.9 (14.3)	87.8 (13.2)	85.7 (11.0)
CVD risk factors			
CVD (%)	5.8	5.9	5.0
Lipid lowering medication (%)	11.2	10.6	9.3
Total cholesterol (mmol/L)	5.7 (1.1)	5.7 (1.1)	5.6 (0.9)
Fasting triglycerides (mmol/L)	1.4 (1.1)	1.3 (0.9)	1.1 (0.6)
HDL cholesterol (mmol/L)	1.5 (0.5)	1.5 (0.4)	1.6 (0.4)

Results are based on analyses weighted toward the BMI distribution of the general population (n=6,360). Data are shown as mean (standard deviation), median [interquartile range] or percentage.

[1]Low education: none, primary school, or lower vocational education as highest level of education.

[2]Proportion menopausal state only estimated in women (n=3,352)

[3]Mean VAT only calculated in persons with VAT measurement (n=2,450)

[4]Mean HTGC only calculated in persons with HTGC measurement (n=1,809)

BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoproteins; MET, metabolic equivalent of task.

Table 2. Difference in measure of body fat with 95% confidence intervals per 10 points higher on the DHD-index in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age

	Total body fat (N=6,360)	**Visceral adipose tissue (N=2,450)**	**Hepatic triglyceride content (N=1,809)**
	Difference in TBF (%) (95% CI)	Difference in VAT (cm^2) (95% CI)	Relative change in HTGC (95% CI)
Crude			
Total	0.5 (0.3; 0.7)	-6.7 (-8.5; -4.9)	0.90 (0.86; 0.94)
Men	-0.6 (-0.8; -0.4)	-5.6 (-8.6; -2.7)	0.93 (0.87; 1.00)
Women	-0.4 (-0.6; -0.2)	-1.6 (-3.5; 0.4)	0.93 (0.87; 0.99)
Multivariable[1]			
Total	-0.2 (-0.3; -0.1)	-3.2 (-4.7; -1.8)	0.92 (0.88; 0.96)
Men	-0.3 (-0.4; -0.1)	-4.3 (-6.9; -1.8)	0.93 (0.88; 1.00)
Women	-0.2 (-0.3; -0.1)	-2.2 (-3.7; -0.6)	0.91 (0.85; 0.96)
Multivariable + TBF			
Total		-2.3 (-3.5; -1.0)	0.94 (0.90; 0.98)
Men		-2.3 (-4.4; -0.2)	0.96 (0.91; 1.00)
Women		-1.4 (-2.8; -0.1)	0.92 (0.87; 0.98)

Results are based on analysis weighted toward the body mass index distribution of the general population.

[1]Adjusted for age, total energy intake, smoking, education, ethnicity, basal metabolic rate, menopause and energy restricted diet

CI, confidence interval; DHDI, Dutch Healthy Diet Index, TBF, total body fat; VAT, visceral adipose tissue.

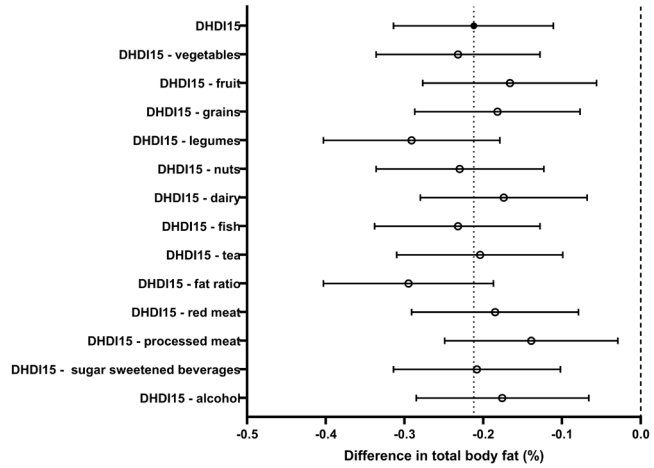

Figure 1. Association between 10 points on the Dutch Healthy Diet Index and total body fat when leaving one component out at the time (N=6,361), adjusted for sex, age, smoking status, education, ethnicity, basal metabolic rate, menopausal state, energy restricted diet, total energy intake and component left out. Results are based on analyses weighted toward the body mass index distribution of the general population. DHDI, Dutch Healthy Diet Index.

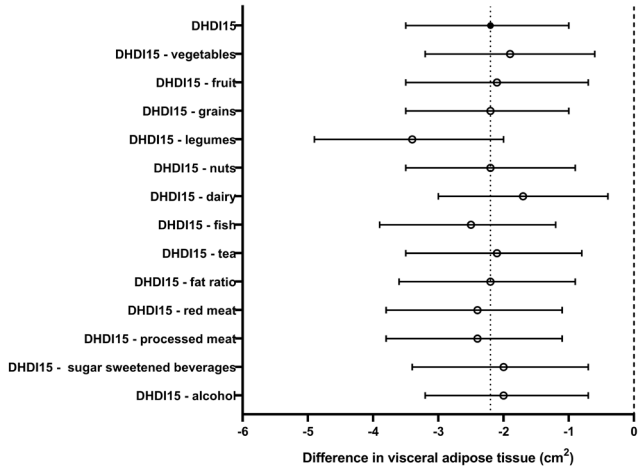

Figure 2. Association between 10 points on the Dutch Healthy Diet Index and visceral adipose tissue when leaving one component out at the time (N=2,449), adjusted for sex, age, smoking status, education, ethnicity, basal metabolic rate, menopausal state, energy restricted diet, total energy intake, total body fat and component left out. Results are based on analyses weighted toward the body mass index distribution of the general population. DHDI, Dutch Healthy Diet Index.

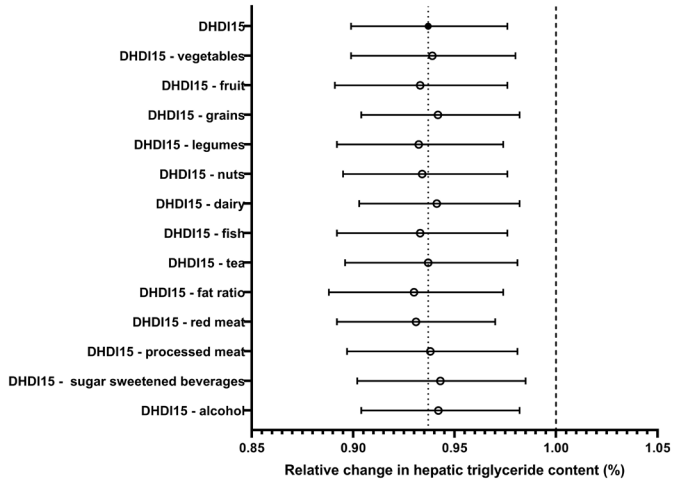

Figure 3. Association between 10 points on the Dutch Healthy Diet Index and hepatic triglyceride content when leaving one component out at the time (N=1,809), adjusted for sex, age, smoking status, education, ethnicity, basal metabolic rate, menopausal state, energy restricted diet, total energy intake, total body fat and component left out. Results are based on analyses weighted toward the body mass index distribution of the general population. DHDI, Dutch Healthy Diet Index.

Dutch Healthy Diet Index in relation to hepatic triglyceride content

After adjustment for potential confounding factors and total body fat, 10 points higher on the DHD-index was associated with less liver fat (0.94 times, 95% CI 0.90; 0.98). (**Table 3**). Leaving out components did not alter the association. Associations were comparable for men and women (data not shown). Associations between the DHD-index and liver fat were comparable in participants with or without obesity (**Supplemental table 1**). When stratified by menopausal state, associations were only present in postmenopausal women. Associations were similar in both carriers and non-carriers of the PNPLA3 risk allele (**Supplemental table 1**).

DISCUSSION

In this population-based study of middle-aged men and women, we aimed to study the association between adherence to the Dutch Guidelines for a Healthy Diet 2015 and total body fat, visceral fat and liver fat, as assessed with bio impedance analysis, MRI and ^{1}H-MRS. After adjustment for potential confounding factors, a higher score on the DHD-index, and therefore a greater adherence, was associated with less total body fat, less visceral fat and less liver fat. Associations with visceral fat and liver fat remained present after adjustment for total body fat, indicating specific associations with visceral fat and liver fat rather than with overall adiposity. When leaving all the thirteen components out one by one to examine which component contributes the most to the association, all components seemed similarly important. No clear overall differences were observed between BMI categories, post- and premenopausal women or between carriers and non-carriers of the PNPLA3 risk allele.

Several previous studies have shown associations between diet quality indices with a moderate protective effect regarding multiple health outcomes, as reduced risks of cardiovascular disease and mortality [15]. In a meta-analysis, adherence to high-quality diets as assessed by the (Alternative) Healthy Eating Index and the Dietary Approaches to Stop Hypertension were associated with decreased risks of all-cause mortality (22%), CVD (22%), cancer (15%) and of type 2 diabetes (22%) [32]. Although the 2015 DHD-index is relatively new, a higher adherence to the 2015 Dutch Guidelines for a Healthy Diet has been associated with a decreased risk of stroke, chronic obstructive pulmonary disease (COPD), colorectal cancer and all-cause mortality [33].

In another recent systematic review on diet quality indices in relation to measures of obesity it has been shown that adherence to the Healthy Eating Index, the Dietary

Guidelines for Americans Index and the Dietary Guideline Index were associated with either lower BMI or waist circumference [34]. Moreover, in a recent meta-analysis, healthy dietary patterns were inversely related to both visceral fat and subcutaneous fat [35], although most studies did not adjust for total body fat and therefore the associations might not be specific for visceral fat and subcutaneous fat. Dietary intake of fiber and calcium was inversely related with visceral fat, and there was a positive relation of unhealthy dinner-type dietary patterns and consumption of alcohol and fructose with visceral fat [35]. Our study contributes to this knowledge by showing that adherence to dietary guidelines for a healthy diet as a whole, was not only associated with total body fat, but also specifically with visceral fat and liver fat. Our findings thereby suggest that next to total body fat, visceral fat and liver fat may mediate the previous observed associations of diet indices with cardiometabolic risk.

Improvement of diet quality in terms of an increase in the (Alternative) Healthy Eating Index 2010, the alternate Mediterranean Diet Score and the Dietary Approaches to Stop Hypertension score has been associated with decreased weight gain, especially in people with a BMI over 25 kg/m^2 [36]. This corresponds with our findings, that showed that the association between the DHD-index and VAT was slightly stronger in people with a BMI over 30 kg/m^2, but not for liver fat.

A study on the HEI-2010 in young Americans has also demonstrated an inverse association with body fatness in men, after taking level of physical activity into account [37]. Physical activity is a component of the DHD-index. However, when leaving out the physical activity component from the DHD-index, the associations attenuated but remained, suggesting the importance of both diet and physical activity in relation to TBF, VAT and HTGC.

Strengths of this study are the sample size and the extensive phenotyping, allowing adjustment for multiple potential confounding factors and investigation of multiple subgroup analyses. Moreover, we directly assessed visceral adipose tissue and hepatic triglyceride by MRI and ^{1}H-MRS in a relatively large subsample of the study population. The Dutch Healthy Diet Index is a measure of adherence to the current (2015) Dutch dietary guidelines and reflects the whole diet as it includes multiple food group based components. Multiple improvements have been made compared to the previous 2006 guidelines and DHD-index. For example, fruit juices are now no longer included in the fruit component bur rather in the sweetened beverages component, and whereas the previous guidelines focused on saturated fat without taking the source into account, the new index includes a component on the solid to liquid fat ratio.

A limitation of our study is that dietary intake of food products is measured with a self-administered FFQ, making it prone to measurement error. Potential social desirability might have overestimated the average score. Although this might have affected associations with total body fat, it is less likely that this would affect associations with visceral fat or liver fat because people are not aware of the amount of visceral fat or liver fat they have. Moreover, total body fat has been estimated using BIA with the Tanita foot-to-foot BIA system. Although it has been suggested that foot-to-foot BIA might give an overestimation of the amount of fat mass [38], another study found a strong correlation (r = 0.84) between foot-to-foot and hand-to-foot BIA with regards to total body fat percentages [18]. Furthermore, a strong correlation (r = 0.89) was also found in a study comparing resistance measurements provided by foot-to-foot BIA with measurements from dual-energy X-ray absorptiometry and underwater weighing [39]. Additionally, the population of this study predominantly consisted of Caucasian, middle-aged participants, so results should be confirmed in other age and ethnic groups. However, a large prospective cohort study conducted in the South-eastern part of the United States, showed that associations between adherence to the Dutch Guidelines for Americans as assessed by the HEI-2010, were similar between African-Americans and whites [40]. As the FFQ used in our study did not contain complete information on certain food items, we had to make several assumptions in order to calculate the 2015 DHD-index. For example, we could not make a distinction between salted and unsalted nuts. As a result, salted nuts and beer nuts are now included in this component, which could have influenced the results. Moreover, the wholegrain component is now only based on breakfast cereals, which may therefore result measurement error. Moreover, the wholegrain component is now only based on breakfast cereals, which may therefore result measurement error. Lastly, the observational cross-sectional design of this study precludes causal inference.

Whereas the associations with visceral fat and liver fat content may seem weak, it must be noted that an increase of 10 points on the DHD-index can be easily accomplished and the results might therefore be considerably relevant in general practice. For example, consumption of one apple and one cup of broccoli per day extra adds up to a 10 points higher DHD-index, which was associated with more than 2 cm^2 less visceral fat. As previous results has shown that visceral fat and liver have been associated with insulin resistance, metabolic risk factors and cardiovascular disease [3-6], adherence to the Dutch Guidelines for a Healthy Diet might ultimately be associated with a decreased risk of developing insulin resistance or cardiovascular disease, although direct and exact translation to disease risk remains difficult.

In conclusion, in this population-based study in middle-aged men and women,

adherence to the Dutch Guidelines for a Healthy Diet from 2015 as assessed by the DHD-index, was associated with less total body fat, but also specifically with less visceral fat and liver fat. These associations do not seem driven by one component in particular, indicating the importance of an overall healthy lifestyle to prevent cardiometabolic disorders. These findings might contribute to better understanding of the mechanisms underlying associations between dietary habits and cardiometabolic diseases. Future intervention studies are therefore needed to assess whether, and to what extent, changes in a person's lifestyle can specifically influence visceral fat and liver fat and thereby reduce the risk of cardiometabolic diseases

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Consumption of Alcoholic and Sugar-Sweetened Beverages is Associated with Increased Liver Fat Content in Middle-Aged Men and Women

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ABSTRACT

Background
Fatty liver is the leading cause of chronic liver diseases and increases the risk of cardiovascular disease. Besides alcohol consumption, energy-containing non-alcoholic beverages may contribute to liver fat accumulation.

Objective
We aimed to study the consumption of alcoholic and non-alcoholic beverages and their mutual replacement in relation to hepatic triglyceride content (HTGC) in middle-aged men and women.

Methods
In this cross-sectional analysis, HTGC was assessed by ^{1}H-MRS. Habitual consumption of alcoholic and non-alcoholic beverages was assessed using a validated food frequency questionnaire. All beverages were converted to standard servings and to percent of total energy intake (En%). We performed linear regression to examine the association of alcoholic and non-alcoholic beverages with HTGC, adjusted for age, sex, smoking, education, ethnicity, physical activity, total energy intake and total body fat. We studied replacement of alcoholic beverages with non-alcoholic beverages per serving/d and per 5 En%/d.

Results
After exclusion of individuals with missing values, 1,966 participants (47% men) were analyzed, with a mean ± SD age of 55 ± 6 years, BMI of 26 ± 4 kg/m^2, and HTGC of 5.7 ± 7.9 %. Each extra alcoholic serving per day was associated with more liver fat (1.09 times, 95% CI: 1.05, 1.12). Replacing 5 En% of alcoholic beverages with milk was associated with less liver fat (0.89 times, 95% CI: 0.81, 0.98), whereas replacement with 5 En% of sugar sweetened beverages was associated with liver fat to a similar extent as the alcoholic beverages (1.00 times, 95% CI: 0.91, 1.09).

Conclusion
In a population-based cohort, consumption of each extra daily alcoholic beverage was associated with more liver fat. In isocaloric replacement, milk was associated with less liver fat, whereas sugar sweetened beverages were equally associated with liver fat. This suggests that intake of alcohol and sugars may contribute to liver fat accumulation.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is often defined as a hepatic triglyceride content of more than 5.56% not due to excessive alcohol consumption [1]. NAFLD covers a broad clinical spectrum, ranging from the most common feature, hepatic steatosis, to non-alcoholic steatohepatitis (NASH), and liver cirrhosis [2], and increases the risk of end-stage liver disease and liver-related and all-cause mortality [3-6]. Although the incidence of NAFLD is underreported and varies widely [7], the prevalence has risen considerably over the last two decades [8] to 14 to 34% of the general population in Europe [9, 10], Asia [11] and the United States of America [7, 11]. The prevalence of NAFLD in obesity might even be as high as 90% [12], possibly due to excessive calorie intake [13]. It is the leading cause of chronic liver diseases worldwide [14], and is also strongly associated with the metabolic syndrome [15] and cardiovascular diseases [16]

Excessive alcohol consumption[17] is a well-established risk factor of both hepatic steatosis (liver fattening) and liver disease. Current guidelines to prevent or reduce liver fat accumulation therefore recommend that heavy alcohol consumption should be discouraged [18]. However, there is much controversy whether moderate alcohol consumption should also be discouraged, as there are studies indicating that light to moderate alcohol consumption might be protective in relation to fatty liver and (extra) hepatic complications [18-23], whereas in a mendelian randomization study it has been suggested there is no beneficial effect of moderate alcohol consumption on the severity of nonalcoholic fatty liver disease [24]. Moreover, it has been shown that liquid food leads to less satiety and more postprandial hunger [25]. Particularly alcohol is very inefficient in activating the satiety mechanism, and consuming alcohol during meals might lead to higher food consumption [26].

In addition, sugar sweetened beverages (SSB), but not diet sodas have been associated with fatty liver [27]. This suggests that energy-containing drinks in general, or specifically dietary sugars may increase liver fat as well [28, 29]. As the relative contributions of different types of non-alcoholic and alcoholic beverages consumption to liver fat accumulation remain unclear, we aimed to directly compare the associations of consumption of alcoholic beverages and non-alcoholic energy-containing and non-energy containing beverages with hepatic triglyceride content in a large sample of the general population. Insight in these associations may contribute to lifestyle guidelines, especially with regard to beverages, for both primary and secondary prevention aims.

METHODS

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based cohort study in 6671 individuals aged 45 to 65 years, with an oversampling of persons with a BMI of 27 kg/m^2 or higher. Men and women aged between 45 and 65 years with a self-reported BMI of 27 kg/m^2 or higher living in the greater area of Leiden (in the West of The Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI, allowing for a reference distribution of BMI. Detailed information about the study design and data collection has been described elsewhere [30].

The present study is a cross-sectional analysis of the baseline measurements of the participants with a measurement of hepatic triglyceride content (HTGC). For our analyses we excluded participants with an implausibly high or low total energy intake (<600kcal or >5000kcal) and missing data on beverage consumption or potential confounding factors. The study was approved by the medical ethics committee of the LUMC and all participants gave written informed consent.

Beverage consumption

Habitual consumption of beverages of all participants was estimated using a semi-quantitative food frequency questionnaire, which was originally designed to study dietary fat intake [31, 32]. Consumption of alcoholic and non-alcoholic beverages was assessed in absolute frequency (times per day, week, month). Participants were asked about consumption of different alcoholic beverages (beer, wine, liquor and mixed drinks (such as cocktails). For each alcoholic beverage we used a standard serving as based on the Dutch Food Composition Database (NEVO-2011): 200 grams for beer, 110 for wine, 50 for liquor and 258 for mixed long drinks so that each consumption contained 10 grams of alcohol. Non-alcoholic beverages were also converted to standard servings: 200 grams for non-alcoholic beers, 125 grams for coffee and tea, 150 grams for milk and 150 grams for sugar-sweetened beverages (NEVO-2011). Non-alcoholic beverages were divided into energy-containing (non-alcoholic beers, milk and sugar sweetened beverages) or non-energy containing (tea and coffee without milk) beverages. No information on water consumption or diet sodas was collected using the FFQ. After the conversion to standard servings, all non-alcoholic beverages were also summed up into one variable. The same was done for all alcoholic beverages. Total alcoholic beverage consumption was divided into subcategories: 0 to 0.5 grams of alcohol per day (g/d) (including abstainers), 0.5 to 5 g/d, 5 to 15 g/d for women and 5-30 g/day for men and lastly >15 g/d for women and >30 g/d for men.

We assessed the reproducibility of the habitual consumption of different beverages in a random subgroup of 100 participants who completed the FFQ for a second time approximately three months after the baseline measurement. The individual measurement intraclass correlation coefficients of the different beverages were 0.63 for sugar sweetened beverages, 0.81 for milk, 0.82 for coffee, 0.91 for tea, 0.79 for beer, 0.82 for wine, 0.67 for mixed drinks and 0.89 for liquor, which can be considered good to excellent[33].

^{1}H-MR spectroscopy of liver fat content

^{1}H-MR spectroscopy of the liver was performed on a 1.5 Tesla whole-body MR scanner (Philips Medical Systems, Best, the Netherlands), and spectra were obtained as described previously [34]. ^{1}H-MRS data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium) [35]. Hepatic triglyceride content relative to water was calculated as (signal amplitude of triglyceride) / (signal amplitude of water) x 100.

Data collection of covariates

In the baseline questionnaire, participants reported smoking behaviour in three categories: current, former or never smoking (reference group). Ethnicity was reported by self-identification in eight categories which we grouped into white (reference group) and other. Highest level of education was reported in 10 categories according to the Dutch education system and grouped into high (including higher vocational school, university, and post-graduate education) versus low education (reference group). Physical activity during leisure time was reported using the Short Questionnaire to Assess Health-enhancing physical activity and was expressed in MET-hours per week (36). Data collection on other covariates has been described previously [30].

Statistical analyses

In the NEO study there is an oversampling of persons with a BMI of 27 kg/m^2 or higher. To correctly represent associations in the general population [37], adjustments for this oversampling were made. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality [38], whose BMI distribution was similar to the BMI distribution of the general Dutch population [39] (**Supplemental table 1**). All results were based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI ≥ 27 kg/m^2. As a result of the weighting, only percentages and proportions can be given instead of numbers of participants. Baseline characteristics are displayed in percentages or means (standard deviations) for the total population, and stratified by sex and categories of alcohol consumption.

We performed linear regression analyses to examine the association between alcohol consumption and liver fat. We performed three different models and also stratified each model by sex, due to the known differences in both alcohol consumption and liver fat content between men and women. Because of the skewed distribution of HTGC, we used the natural logarithm of this variable in the analyses. For an easier interpretation of these results, we back transformed the regression coefficients towards a ratio (using exp(beta)) with 95% confidence interval. Such ratio, for example 1.2, can be interpreted as 1.2 times HTGC for each extra serving per day, which would reflect an increase in HTGC from, for example, 5% to 6%.We first performed linear regression analysis to examine the association of alcohol consumption as a categorical variable (0 to 0.5 g/d (reference),≥0.5 to 5 g/d, ≥5 to 15 g/d for women and ≥5 to 30 g/d for men, and ≥15 g/d for women and≥30 g/d for men) with HTGC. We tested for a linear trend (p=0.01) and also added a quadratic term (p=0.49) to the model to check for non-linearity.

Then, we studied alcohol consumption as a continuous outcome in three different ways. Firstly, we studied the association between one serving of alcohol (total alcohol, beer, wine, mixed drinks and liquor) and one serving of non-alcoholic beverages (sugar sweetened beverages, milk, coffee, tea and non-alcoholic beer) per day and liver fat content. This was done in both a crude model and a multivariable linear regression model, which was adjusted for age, sex, smoking, education, ethnicity, physical activity and total energy intake. Models studying separate alcoholic beverages were additionally adjusted for all other alcoholic beverages, and models on non-alcoholic beverages were additionally adjusted for all other non-alcoholic beverages.

Secondly, we studied the effect of substituting one serving of an alcoholic beverage with one serving of a non-alcoholic beverage. In these substitution models we included a sum variable of all beverages, in addition to each beverage separately, except for the beverage to be substituted, in this case alcoholic beverages. Instead of total energy intake, these substitution models were adjusted for caloric intake from food only, to adjust for possible confounding when substituting different beverages. Accordingly, the regression coefficients can be interpreted as the relative change in HTGC if one serving/d of an alcoholic beverage was substituted by one serving/d of a non-alcoholic beverage.

Third, in addition to the substitution analyses based on servings, we also performed an isocaloric substitution model of alcoholic beverages with energy-containing non-alcoholic beverages. This model was adjusted for both caloric intake from beverages and caloric intake from food. In these analyses 5 En% of alcoholic beverages is replaced with 5 En% of energy containing non-alcoholic beverages (sugar-sweetened beverages, milk, and non-alcoholic beer) in relation to HTGC, to examine to what extent the caloric content contributes to liver fat content. To study whether the associations are specific for liver fat, we additionally adjusted all three models for total body fat.

To examine to what extent consumption of alcoholic beverages was associated with liver fat content in participants without a fatty liver, we stratified the analyses by the arbitrary cut-off point of 5.56% which indicates a fatty liver. Next to that, we stratified by the rs738409 single nucleotide polymorphism (SNP) in the *PNPLA3* gene that is associated with diffuse fat deposition in the liver and may promote NASH, fibrosis and cirrhosis throughout the liver [40], to investigate whether the associations differ between carriers and non-carriers of the SNP.

As a means of sensitivity analysis, we repeated the substitution analysis based on servings after taking into account the milk and sugar potentially added to coffee and tea. In the analyses with categories of alcohol consumption, we repeated the analyses after excluding alcohol abstainers (0 g/d) from the reference group. Additionally, we performed the models after exclusion of participants with diabetes type 2 or cardiovascular disease, as they might have changed their drinking habits after being diagnosed, or might potentially react differently to sugars.

All above mentioned analyses were pre-defined, and analyses not pre-specified are considered exploratory. We performed all analyses using STATA statistical Software (Statacorp, College Station, Texas, USA), version 14.

RESULTS

In total, 6,671 participants were included in the NEO study between September 2008 and October 2012, of whom 2,580 underwent a liver fat measurement by ^{1}H-MRS. However, due to the limited time slot that was available per participants did not allow time for a repeat examination when technical failures were present (n=497), leaving 2,083 participants with a successful liver fat measurement. After exclusion of participants with extreme energy intake (n=18), missing dietary data (n=26), missing data on potential confounding factors (n=1 for smoking, n=16 for education, n=2 for ethnicity, n=44 for physical activity in leisure time, n=3 for total body fat and n=6 for visceral adipose tissue) and one participant for whom the log transformation of the liver fat could not be calculated, 1,966 participants were included in the analyses. Baseline characteristics of these participants are presented in **table 1**. Participants with higher

Table 1. Characteristics of participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by [1]H-MRS [1]

				Alcohol consumption			
	Total population	**Men (47%)**	**Women (53%)**	**0-0.5 g/d (16%)**	**>0.5-5 g/d (21%)**	**>5-15 g/d women >5-30 g/d men (38%)**	**>15 g/d women >30 g/d men (24%)**
Age (year)	55 ± 6	56 ± 6	55 ± 6	55 ± 7	55 ± 6	56 ± 6	56 ± 6
Sex (% men)	47			30	34	60	44
Ethnicity (% white)	96	96	96	92	93	97	99
Education (% high)	46	51	42	32	41	50	55
Smoking (% current)	14	16	13	13	7	13	23
Physical activity in leisure time (MET h/wk)	37.8 ± 31.9	39.1 ± 37.1	36.7 ± 27.3	38.6 ± 38.4	35.3 ± 28.0	38.4 ± 31.9	38.9 ± 31.4
Sugar sweetened beverages (serving/d)[2]	0.8 ± 1.0	0.9 ± 1.1	0.8 ± 0.9	1.1 ± 1.4	0.9 ± 1.0	0.8 ± 0.8	0.8 ± 0.9
Milk (serving/d)[2]	1.1 ± 1.0	1.2 ± 1.2	0.9 ± 0.9	1.1 ± 1.2	1.1 ± 1.0	1.0 ± 0.9	1.0 ± 1.1
Coffee (serving/d)[2]	3.7 ± 2.1	4.3 ± 2.4	3.2 ± 1.8	3.3 ± 2.4	3.1 ± 2.0	4.0 ± 2.1	4.0 ± 2.1
Tea (serving/d)[2]	2.0 ± 1.9	1.4 ± 1.7	2.4 ± 2.0	2.1 ± 2.2	2.3 ± 1.9	1.8 ± 1.7	1.7 ± 2.1
Non-alcoholic beer (serving/d)[2]	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	0.0 ± 0.2	0.0 ± 0.1	0.0 ± 0.2	0.0 ± 0.1
CVD (%)	5	5	4	8	4	3	5
Diabetes (%)	3	4	2	7	2	2	3
BMI (kg/m^2)	25.9 ± 3.9	26.6 ± 3.5	25.2 ± 4.0	26.5 ± 5.0	25.6 ± 4.1	25.8 ± 3.3	25.9 ± 4.1
Waist circumference (cm)	91.0 ± 12.6	97.5 ± 10.7	85.4 ± 11.3	91.0 ± 15.1	89.6 ± 11.8	91.4 ± 11.6	91.5 ± 13.7
Total body fat (%)	30.7 ± 8.2	24.6 ± 5.7	36.1 ± 6.0	34.3 ± 8.8	31.9 ± 8.6	28.6 ± 7.6	31.3 ± 7.7
VAT (cm^2)	88.6 ± 55.1	114.5 ± 60.0	65.9 ± 39.8	85.1 ± 60.0	81.5 ± 49.5	89.9 ± 52.2	95.0 ± 61.3
HTGC (%)	5.7 (7.9)	7.0 (8.3)	4.6 (7.3)	5.4 (8.2)	5.0 (7.4)	5.5 (6.9)	6.8 (9.5)
HTGC>5.56% (%)	29	39	21	25	24	29	35
Fasting serum triglycerides (mmol/L)	1.2 (0.8)	1.4 (1.0)	1.1 (0.7)	1.2 (0.8)	1.2 (0.8)	1.3 (0.8)	1.3 (1.0)

[1]Values are means ± SDs or percentage. Results are based on analyses weighted toward the BMI distribution of the general population (*n*=1,966). [2]Servings equal 150 grams for sugar-sweetened beverages, 150 grams for milk, 125 grams for coffee and tea and 200 grams for non-alcoholic beers. BMI, body mass index; CVD, cardiovascular disease, HTGC; hepatic triglyceride content, MET, metabolic equivalent of task; VAT, visceral adipose tissue.

alcohol consumption were more often smokers and had on average a higher education. Hepatic triglyceride content and the prevalence of a fatty liver was also higher in the categories with higher alcohol consumption. Whereas men on average have a higher coffee and beer consumption, women have a higher tea consumption.

Table 2 displays the association between different categories of alcohol consumption and liver fat content. Despite a linear trend (P for trend 0.01), light and moderate consumption were not significantly associated with liver fat (Table 2). Compared with no alcohol consumption (0-0.5 g/d), high alcohol consumption (>15 g/d for women and >30 g/d for men) was associated with more liver fat, for total alcohol consumption (1.28 times, 95% CI: 1.06, 1.55), beer consumption (1.39 times, 95% CI: 1.08, 1.80) and wine consumption (1.28 times, 95% CI: 1.04, 1.58) (Table 2). Results were similar when excluding alcohol abstainers (0g/d) from the reference group (data not shown).

Table 3 shows the associations between consumption of different alcoholic beverages as continuous variables and liver fat content. Each extra alcoholic serving was associated with more liver fat (1.09 times, 95% CI: 1.06; 1.13). When additionally adjusted for total body fat to examine whether the associations were specific for liver fat, associations attenuated for liquor and mixed drinks, although total alcoholic beverages remained associated with more liver fat (1.09 times, 95% CI: 1.05, 1.12).

The associations of non-alcoholic beverages are shown in **table 4.** In the total population, each extra serving of non-alcoholic beverages was associated with less liver fat (0.97 times, 95% CI: 0.95, 0.99). Consumption of coffee (0.96 times for each extra serving, 95% CI: 0.93, 0.99), tea (0.97 times, 95% CI: 0.94; 1.00) and milk (0.95 times, 95% CI: 0.89; 1.00) was also associated with less liver fat. Results did not differ after exclusion of participants with diabetes type 2 or cardiovascular disease or when taking the milk and sugar added to coffee and tea into account (data not shown).

Table 5 shows that substituting one alcoholic serving with one non-alcoholic serving was associated with less liver fat (0.90 times, 95% CI: 0.86, 0.94) in the total population after adjustment for potential confounding factors and total body fat. Of the different non-alcoholic beverages, replacement with milk (0.88 times, 95% CI: 0.82, 0.95), tea (0.89 times; 95% CI: 0.85; 0.94) and coffee (0.88 times, 95% CI: 0.84; 0.92) was associated with less liver fat. Results were similar when taking the milk and sugar added to coffee and tea into account (data not shown).

Isocaloric substitution of 5 En% of alcoholic beverages with 5 En% of non-alcoholic beverages (**table 6**) showed that substitution of alcohol with milk was associated with less HTGC (0.89 times, 95% CI: 0.81, 0.98) in the total population. Replacing 5 En% of alcohol with 5 En% of sugar sweetened beverage was associated with liver fat equally strong as with alcohol (1.00 times, 95% CI: 0.91, 1.09).

Table 2. Relative change in HTGC and 95% confidence intervals for different categories of alcohol consumption in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by [1]H-MRS

	0 to 0.5 g/d	≥0.5 to 5 g/d	≥5 to 15 g/d women ≥5 to 30 g/d men	≥15 g/d women ≥30 g/d men	*P*-trend
Alcohol (total)					
Multivariable-adjusted[1]relative change (95% CI)	1 (ref)	1.05 (0.87, 1.25)	1.07 (0.90, 1.28)	1.28 (1.06, 1.55)	**0.01**
Proportion of population, %	13.7	22.2	41.0	23.1	
Beer[2]					
Multivariable-adjusted relative change (95% CI)	1 (ref)	0.94 (0.83, 1.08)	1.10 (0.93, 1.29)	1.39 (1.08, 1.80)	**0.03**
%	48.1	27.5	18.5	6.0	
Wine[2]					
Multivariable-adjusted relative change (95% CI)	1 (ref)	1.01 (0.88, 1.16)	1.02 (0.89, 1.18)	1.28 (1.04, 1.58)	**0.16**
%	23.3	32.7	34.8	9.3	

[1]Adjusted for age, sex, smoking, education, ethnicity, physical activity in leisure time, total energy intake and total body fat. Results are based on analyses weighted towards the body mass index distribution of the general population (*n*=1,966), and derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as a relative change compared with the reference category. Such ratio, for example 1.2, can be interpreted as 1.2 times HTGC for each extra serving per day, which would reflect an increase in HTGC from, for example, 5% to 6%.CI, confidence interval; HTGC, hepatic triglyceride content.

[2]Additionally adjusted for other alcoholic beverages. Servings equal 200 grams for beer and 110 grams for wine.

Table 3. Relative change in HTGC and 95% confidence intervals per 1 serving/d higher consumption of alcoholic beverage in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by [1]H-MRS[1]

	Crude	Multivariable	Multivariable + TBF
	Relative change (95% CI)	Relative change (95% CI)	Relative change (95% CI)
Alcohol (total)			
Total	1.15 (1.11; 1.19)	1.09 (1.06; 1.13)	1.09 (1.05; 1.12)
Men	1.11 (1.07; 1.15)	1.11 (1.07; 1.15)	1.09 (1.05; 1.13)
Women	1.05 (1.07; 1.15)	1.09 (1.00; 1.18)	1.10 (1.02; 1.19)
Beer[2]			
Total	1.14 (1.09; 1.19)	1.07 (1.02; 1.11)	1.08 (1.03; 1.13)
Men	1.08 (1.04; 1.13)	1.08 (1.04; 1.13)	1.09 (1.04; 1.15)
Women	0.95 (0.84; 1.07)	1.02 (0.89; 1.17)	1.06 (0.96; 1.17)
Wine[2]			
Total	1.13 (1.06; 1.20)	1.13 (1.06; 1.21)	1.11 (1.05; 1.18)
Men	1.13 (1.06; 1.21)	1.13 (1.06; 1.21)	1.08 (1.02; 1.15)
Women	1.12 (1.02; 1.24)	1.13 (1.02; 1.26)	1.15 (1.04; 1.28)
Liquor[2]			
Total	1.64 (1.45; 1.86)	1.22 (1.08; 1.38)	1.06 (0.93; 1.21)
Men	1.33 (1.17; 1.50)	1.24 (1.10; 1.40)	1.10 (0.97; 1.26)
Women	1.28 (0.66; 2.48)	0.95 (0.52; 1.73)	0.62 (0.37; 1.04)
Mixed drinks[2]			
Total	1.56 (1.34; 1.83)	1.18 (1.00; 1.40)	0.97 (0.83; 1.15)
Men	1.26 (1.09; 1.47)	1.18 (1.00; 1.40)	0.98 (0.83; 1.16)
Women	1.74 (0.95; 3.20)	1.53 (0.86; 2.71)	1.12 (0.63; 1.97)

After stratifying the analyses by the cut-off point of fatty liver (HTGC>5.56%), associations between alcohol consumption and liver fat were similar in both groups (**Supplemental table 2**). Regarding the *PNPLA3* polymorphism, the association between each alcoholic beverage and HTGC was similar in both groups (1.14 times for each alcoholic serving extra; 95% CI: 1.07, 1.21 for GC and GG carriers and 1.09 times; 95% CI: 1.04, 1.15 for CC carriers).

Table 4. Relative change in HTGC and 95% confidence intervals per 1 serving/d higher consumption of non-alcoholic beverages in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by [1]H-MRS[1]

	Crude	Multivariable	Multivariable + TBF
	Relative change (95% CI)	Relative change (95% CI)	Relative change (95% CI)
Non-alcoholic beverages (total)			
Total	0.98 (0.95; 1.00)	0.97 (0.94; 0.99)	0.97 (0.95; 0.99)
Men	0.98 (0.95; 1.01)	0.99 (0.95; 1.02)	0.98 (0.95; 1.01)
Women	0.95 (0.91; 0.98)	0.94 (0.91; 0.97)	0.95 (0.92; 0.98)
SSB[2]			
Total	1.07 (1.01; 1.14)	1.05 (0.99; 1.11)	1.03 (0.98; 1.08)
Men	1.04 (0.97; 1.12)	1.07 (0.99; 1.15)	1.02 (0.96; 1.09)
Women	1.05 (0.96; 1.14)	1.02 (0.93; 1.11)	1.03 (0.95; 1.11)
Milk[2]			
Total	1.00 (0.94; 1.07)	0.94 (0.88; 1.00)	0.95 (0.89; 1.00)
Men	0.92 (0.86; 1.00)	0.91 (0.84; 0.99)	0.92 (0.86; 0.99)
Women	1.02 (0.91; 1.13)	0.96 (0.87; 1.07)	0.97 (0.89; 1.06)
Coffee (without sugar or milk)[2]			
Total	1.01 (0.98; 1.04)	0.96 (0.93; 0.99)	0.96 (0.93; 0.99)
Men	1.00 (0.96; 1.04)	0.99 (0.95; 1.03)	0.99 (0.95; 1.02)
Women	0.95 (0.91; 0.99)	0.92 (0.88; 0.96)	0.92 (0.88; 0.96)
Tea (without sugar or milk)[2]			
Total	0.92 (0.89; 0.95)	0.96 (0.92; 0.99)	0.97 (0.94; 1.00)
Men	0.96 (0.92; 1.02)	0.98 (0.93; 1.03)	1.00 (0.94; 1.05)
Women	0.95 (0.92; 0.99)	0.94 (0.90; 0.98)	0.95 (0.91; 0.99)
Non-alcoholic beer[2]			
Total	1.35 (0.99; 1.84)	1.13 (0.88; 1.45)	1.09 (0.86; 0.99)
Men	1.22 0.90; 1.65)	1.18 (0.89; 1.57)	1.17 (0.90; 1.52)
Women	0.82 (0.40; 1.70)	0.88 (0.49; 1.59)	0.73 (0.49; 1.10)

[1]Multivariable: adjusted for age, sex, smoking, education, ethnicity, physical activity in leisure time and total energy intake. Results are based on analyses weighted towards the body mass index distribution of the general population (*n*=1,966), and derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as a relative change. Such ratio, for example 1.2, can be interpreted as 1.2 times HTGC for each extra serving per day, which would reflect an increase in HTGC from, for example, 5% to 6%. CI, confidence interval; HTGC, hepatic triglyceride content; TBF, total body fat.

[2]Additionally adjusted for all other non-alcoholic beverages. Servings equal 150 grams for SSB, 150 grams for milk, 125 grams for tea and coffee, and 200 grams for non-alcoholic beer.

Table 5. Relative change in HTGC and 95% confidence intervals per 1 serving/d of alcoholic beverage substitution by non-alcoholic beverage in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by ^{1}H-MRS[1]

	Crude	Multivariable	Multivariable + TBF
	Relative change (95% CI)	Relative change (95% CI)	Relative change (95% CI)
Non-alcoholic beverages (total)			
Total	0.85 (0.81; 0.88)	0.89 (0.85; 0.93)	0.90 (0.86; 0.94)
Men	0.89 (0.85; 0.93)	0.90 (0.86; 0.95)	0.91 (0.87; 0.96)
Women	0.91 (0.83; 0.99)	0.87 (0.79; 0.95)	0.86 (0.80; 0.94)
Tea (without sugar or milk)[1]			
Total	0.81 (0.78; 0.85)	0.87 (0.83; 0.92)	0.89 (0.85; 0.94)
Men	0.88 (0.82; 0.94)	0.90 (0.84; 0.96)	0.92 (0.86; 0.99)
Women	0.88 (0.79; 0.97)	0.86 (0.78; 0.94)	0.86 (0.79; 0.94)
Coffee (without sugar or milk)[1]			
Total	0.85 (0.81; 0.89)	0.88 (0.84; 0.92)	0.88 (0.84; 0.92)
Men	0.89 (0.84; 0.94)	0.90 (0.85; 0.95)	0.91 (0.86; 0.96)
Women	0.88 (0.79; 0.97)	0.84 (0.76; 0.92)	0.83 (0.77; 0.91)
Milk[1]			
Total	0.86 (0.80; 0.92)	0.87 (0.81; 0.93)	0.88 (0.83; 0.94)
Men	0.83 (0.77; 0.91)	0.84 (0.77; 0.91)	0.86 (0.80; 0.92)
Women	0.94 (0.82; 1.09)	0.90 (0.78; 1.03)	0.89 (0.79; 1.00)
SSB[1]			
Total	0.93 (0.87; 1.00)	0.98 (0.92; 1.04)	0.96 (0.91; 1.02)
Men	0.96 (0.89; 1.03)	1.00 (0.92; 1.08)	0.96 (0.90; 1.03)
Women	0.99 (0.88; 1.11)	0.95 (0.84; 1.06)	0.94 (0.85; 1.04)

[1]Multivariable: adjusted for age, sex, smoking, education, ethnicity, physical activity in leisure time, total energy intake of food, a sum variable of all beverages and all beverages except for alcohol and itself. Results are based on analyses weighted towards the body mass index distribution of the general population (*n*=1,966), and derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as a relative change. Such ratio, for example 1.2, can be interpreted as 1.2 times HTGC for each extra serving per day, which would reflect an increase in HTGC from, for example, 5% to 6%.CI, confidence interval; HTGC, hepatic triglyceride content; SSB, sugar sweetened beverages; TBF, total body fat.

[1]Servings equal 125 grams for tea and coffee, 150 grams for milk and 150 grams for SSB.

Table 6. Relative change in HTGC and 95% confidence intervals per 5 En% of alcoholic beverage substitution by non-alcoholic beverages in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by ^{1}H-MRS[1]

	Crude	Multivariable	Multivariable + TBF
	Relative change (95% CI)	Relative change (95% CI)	Relative change (95% CI)
Non-alcoholic beverages (total)			
Total	0.91 (0.83; 0.98)	0.94 (0.87; 1.02)	0.94 (0.87; 1.01)
Men	0.90 (0.81; 1.00)	0.90 (0.81; 1.01)	0.88 (0.81; 0.97)
Women	0.99 (0.86; 1.14)	0.96 (0.84; 1.10)	0.96 (0.85; 1.09)
Milk			
Total	0.86 (0.77; 0.97)	0.88 (0.79; 0.98)	0.89 (0.81; 0.98)
Men	0.82 (0.71; 0.95)	0.80 (0.69; 0.92)	0.82 (0.72; 0.93)
Women	0.98 (0.82; 1.17)	0.94 (0.79; 1.13)	0.94 (0.81; 1.10)
SSB			
Total	0.95 (0.85; 1.06)	1.01 (0.91; 1.12)	1.00 (0.91; 1.09)
Men	1.00 (0.86; 1.17)	1.04 (0.89; 1.22)	0.97 (0.85; 1.10)
Women	1.01 (0.87; 1.19)	0.98 (0.85; 1.14)	0.99 (0.86; 1.13)

[1]Multivariable: adjusted for age, sex, smoking, education, ethnicity, physical activity in leisure time, total energy intake of beverages, total energy intake from food, and all beverages except for alcohol and itself. Results are based on analyses weighted towards the body mass index distribution of the general population (***n***=1,966), and derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as a relative change. Such ratio, for example 1.2, can be interpreted as 1.2 times HTGC for each extra serving per day, which would reflect an increase in HTGC from, for example, 5% to 6%. CI, confidence interval; HTGC, hepatic triglyceride content; SSB, sugar sweetened beverage; TBF, total body fat.

DISCUSSION

In this population-based cohort of 1,966 middle aged men and women with directly assessed liver fat content, consumption of each extra alcoholic serving per day was associated with more liver fat, with larger increases in liver fat with excessive alcohol consumption. Replacing one alcoholic beverage by one non-alcoholic beverage was associated with less liver fat. Whereas isocaloric replacement of alcohol with milk was associated with less liver fat, isocaloric replacement with sugar sweetened beverages was equally associated with liver fat.

This study was conducted within a large cohort study, in which hepatic triglyceride content has been directly assessed by ^{1}H-MRS. We used substitution analysis to directly compare different types of beverages and their association with liver fat to each other. The comparative nature of our study can contribute to translation to recommendations in clinical practice, as we have shown that consumption of both alcohol and sugar sweetened beverages is associated with more liver fat, whereas milk, tea and coffee are associated with less liver fat. More importantly, replacing alcohol with sugar sweetened beverages is therefore equally associated with liver fat, and replacing it with milk, tea

or coffee is associated with less liver fat. This can be translated into clear advice for patients diagnosed with fatty liver and who are advised to stop consuming alcohol. Lastly, extensive phenotype measurements have been performed, allowing adjustment for many potential confounding factors. However, inherent to the observational, cross-sectional design we cannot exclude residual confounding by lifestyle factors.

Due to the cross-sectional design, a limitation of this substitution analysis is that it is modelled on a group level rather than on an individual level. All participants completed a semi-quantitative FFQ, based on which we estimated the habitual beverage consumption. Although alcohol consumption might have been misreported, intra-class correlations of the beverages showed good to excellent reproducibility. Moreover, by adjusting our analyses for total energy intake we partly corrected for potential misreporting. A limitation of the FFQ is that it did not take drinking habits into account, so we cannot make any statements on the potential role of drinking patterns. Also no information on diet sodas or water was available, so no statements on these beverages can be made. Nevertheless, this will not have influenced the isocaloric substitution models, as only energy-containing beverages were taken into account in this analysis. Results from these isocaloric substitution models suggest that it is not energy per se, but possibly sugars that contribute to liver fat accumulation.

Alcohol is mainly metabolized in the liver [41], and can induce fatty liver by increasing the fatty acid synthesis in the liver. Together with the impaired oxidation of these compounds caused by an increased accumulation of the reduced form of nicotinamide adenine dinucleotice (NADH), alcohol consumption may lead to increased triglyceride synthesis, which is the main form of stored fat stored in the liver [42]. Although many studies investigated the association between light to moderate alcohol consumption and liver fat, results have been inconsistent and inconclusive, and the exact mechanism remains unidentified. A prospective randomized study concluded that moderate red wine consumption during three months increased HTGC in subjects without steatosis at baseline [43], whereas red wine consumption during four weeks in another randomized controlled trial did not significantly increase liver fat compared to de-alcoholized red wine [44]. Additionally, Ekstedt et al. concluded from their long-term follow-up study that moderate alcohol consumption was associated with fibrosis progression in patients with NAFLD and that they should be advised to refrain from heavy episodic drinking [45]. Modest wine consumption has been associated with reduced prevalence of suspected (NA)FLD in other studies [19, 21, 46, 47]. In another study, light to moderate alcohol consumption had a potentially protective effect against insulin resistance in severely obese patients, but not on the severity of activity and stage of liver disease [48]. Although in a recent review an association between moderate alcohol consumption and decreased NASH and fibrosis was shown, it was also observed that heavy episodic drinking may accelerate fibrosis progression [49]. Most of the studies on alcohol consumption, however, including ours, did not take drinking habits into account, only habitual total amount of alcohol consumed. However, even though certain drinking patterns such as drinking outside mealtimes and drinking multiple different alcoholic beverages lead to an increased risk of developing alcohol related liver damage [50], it seems to be the cumulative consumption that is most strongly associated with the progression of alcoholic fatty liver disease [42]. Although current literature is in disagreement about the role of moderate alcohol consumption, none of these studies performed substitution analysis to take into account that a person does not simply stop drinking alcohol but may replace the alcoholic beverages with other drinks. Moreover, results from a recent mendelian randomization suggest that there is no beneficial effect of moderate alcohol consumption on the severity of non-alcoholic fatty liver disease [24]. In our study, light and moderate alcohol consumption were not associated with less liver fat, which is in line with these findings.

Additionally, isocaloric replacement of alcohol with milk was associated with less liver fat in our study. This indicates that it is not caloric intake per se that leads to liver fat accumulation. The exact mechanism behind the seemingly negative association between dairy and liver fat remains unknown, although it is in agreement with current literature. Established biomarkers of dietary dairy fat intake have been associated with higher hepatic and systemic insulin sensitivity, lower fasting glucose concentrations and less liver fat [51]. Moreover, higher low-fat fermented dairy product consumption has also been associated with a decreased risk of developing type 2 diabetes in a prospective study [52].

Importantly, isocaloric replacement of alcohol with sugar sweetened beverage consumption was equally associated with liver fat. Taken together with our results on substitution with milk, this suggest a role for sugars in liver fat accumulation. Our results are in line with recent findings from the Framingham Heart Study that showed a significant dose-response relationship between sugar sweetened beverages and fatty liver disease, but not for diet soda intake [27]. However, replacement of sugar sweetened beverages with other beverages was not investigated in this study.

Multiple underlying mechanisms have been proposed through which sugar sweetened beverages might contribute to the development of diabetes and cardiometabolic diseases not only via overall weight gain, but also independently through the metabolic

effects of constituent sugars [53]. It has also been suggested that liquid foods lead to less satiety and more postprandial hunger [25]. Consumption of sugar sweetened beverages have been shown to induce peaks in blood glucose and insulin levels, contributing to a high glycaemic state, in turn associated with insulin resistance, diabetes and coronary heart disease [53, 54]. In the Netherlands, soft drinks are one of the main sources of fructose [55], which is mostly metabolized to lipids in the liver and might therefore lead to an increase in hepatic de novo lipogenesis [56, 57]. In a recent trial, moderate fructose consumption for 12 weeks increased liver fat despite only a small increase in weight and waist circumference [58]. Moreover, chronic fructose consumption has been shown the decrease resting energy expenditure in a 10-week trial [59]. Our results support the current literature and suggest that both alcoholic beverages and sugar sweetened beverages may contribute to liver fat accumulation. However, in clinical practice, patients with NAFLD are often advised not to consume alcoholic beverages [18] there are no clear guidelines about what they should replace these beverages with.

In conclusion, consumption of alcoholic beverages was associated with a higher liver fat content in a population-based cohort. Replacing a serving of alcoholic beverages with non-alcoholic beverages was associated with less liver fat. Importantly, in isocaloric replacement of alcoholic beverages, sugar sweetened beverages were equally associated with liver fat as alcoholic beverages, suggesting that both alcohol and sugars may contribute to liver fat accumulation. Although intervention studies should confirm to what extent hepatic triglyceride content can actually be changed by altering drinking habits, it is advised to specify with what beverages alcoholic beverages should be replaced in clinical practice, such as non-energy containing beverages or milk, but not with sugar sweetened beverages.

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SUPPLEMENTARY FILES

Supplemental table 1. Body Mass Index distribution of participants of the Netherlands Epidemiology of Obesity study from the Leiderdorp municipality and the resulting weighting factors for the different BMI categories as used in the statistical analyses

Body Mass Index	*n* (%)	Weighting factor
≥30 kg/m²	268 (16.0)	1
≥29-30 kg/m²	83 (5.0)	1.304461
≥28-29 kg/m²	103 (6.2)	1.472934
≥27-28 kg/m²	151 (9.0)	2.458912
≥26-27 kg/m²	172 (10.3)	4.445434
≥25-26 kg/m²	190 (11.4)	8.668198
<25 kg/m²	704 (42.0)	10.26279
Total	**1,671**	

Supplemental table 2. Relative change in HTGC and 95% confidence intervals in stratifications on HTGC, alcohol consumption and PNPLA3 gene in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by ^{1}H-MRS[1]

	Multivariable	Multivariable + TBF
	Relative change (95% CI)	Relative change (95% CI)
Alcohol (total) - per 1 unit/day higher consumption		
HTGC<5.56%	1.05 (1.01; 1.09)	1.04 (1.00; 1.08)
HTGC≥5.56%	1.01 (0.99; 1.04)	1.02 (1.00; 1.05)
PNPLA3 II	1.09 (1.04; 1.15)	1.06 (1.02; 1.11)
PNPLA3 IM+MM	1.14 (1.07; 1.21)	1.12 (1.06; 1.19)
Non-alcoholic (total) - per 1 unit/day higher consumption		
HTGC<5.56%	0.97 (0.95; 0.99)	0.97 (0.95; 0.99)
HTGC≥5.56%	1.00 (0.98; 1.02)	1.00 (0.98; 1.02)
PNPLA3 II	0.96 (0.92; 0.99)	0.95 (0.92; 0.98)
PNPLA3 IM+MM	0.99 (0.95; 1.03)	0.99 (0.96; 1.03)
Non-alcoholic (total) - per 1 unit/day of alcoholic beverage substitution		
HTGC<5.56%	0.93 (0.89; 0.97)	0.94 (0.90; 0.97)
HTGC≥5.56%	0.99 (0.95; 1.02)	0.98 (0.95; 1.01)
PNPLA3 II	0.89 (0.84; 0.95)	0.91 (0.87; 0.96)
PNPLA3 IM+MM	0.87 (0.81; 0.93)	0.89 (0.83; 0.95)
Non-alcoholic (total) - per 5 En% of alcoholic beverage substitution		
HTGC<5.56%	0.94 (0.88; 1.01)	0.95 (0.90; 1.02)
HTGC≥5.56%	0.98 (0.91; 1.06)	0.97 (0.90; 1.04)
PNPLA3 II	0.98 (0.88; 1.08)	0.99 (0.90; 1.08)
PNPLA3 IM+MM	0.87 (0.74; 1.01)	0.87; 0.77; 0.99)

6

Reallocating sedentary time to moderate to vigorous physical activity is associated with reduced total body fat, visceral fat and liver fat

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ABSTRACT

Background

It is unclear how habitual physical activity and sedentary time are associated with the amount of visceral fat and liver fat. We therefore aimed to study substitution of sedentary time with other daily activities and total body fat (TBF), visceral adipose tissue (VAT) and hepatic triglyceride content (HTGC) in middle-aged men and women.

Methods

In this cross-sectional analysis of the NEO study, we objectively assessed physical activity in 932 participants using a combined heart rate monitor and accelerometer (Actiheart). Of those with a valid physical activity measurement, total body fat was assessed by bio impedance balance, VAT by magnetic resonance imaging (MRI) in 317 participants, and HTGC by proton-MR spectroscopy in 265 participants. Activities were categorized as sedentary time, light physical activity (LPA) or moderate to vigorous physical activity (MVPA) and expressed in blocks of 30 minutes. We performed isotemporal substitution analyses adjusted for sex, age, ethnicity, education, Dutch Healthy Diet index, and smoking to estimate associations of replacing 30 minutes/day of sedentary time with 30 minutes/day of other activities.

Results

Participants (41% men) had a mean (SD) age of 56 (6) years and performed 82 (55) minutes of MVPA and spent 9.1 hours (2.1) sedentary per day. Replacing 30 minutes/day of sedentary time with 30 minutes of MVPA was associated with 0.5% less TBF (95% CI: -0.9, -0.1), 7.2 cm2 less VAT (-10.7, -3.6) and with less HTGC (0.89 times; 0.82, 0.97). Replacement with LPA was not associated with TBF (-0.17 %; -0.45, 0.11), VAT (0.4 cm2; -2.1, 2.9) or HTGC (0.98; 0.92, 1.04).

Conclusions

Replacing sedentary time with MVPA, but not with LPA, was negatively associated with total body fat, and visceral and liver fat. These findings contribute to the development of more specified guidelines on sedentary time and physical activity.

INTRODUCTION

Abdominal obesity is a well-established risk factor for diabetes mellitus, the metabolic syndrome and cardiovascular diseases [1,2]. In particular accumulation of fat in the visceral area and in and around the organs [2], such as the liver, carries extra risk. Both visceral fat and liver fat have been associated with metabolic risk factors, insulin resistance and cardiovascular diseases [3-6], making these adipose depots important targets to prevent cardio metabolic diseases.

Meta-analyses have shown that exercise can reduce both visceral fat [7] and liver fat [8]. However, the included studies focused on structured exercise rather than habitual daily activities and sedentary time, and evidence on the association between habitual daily activity and different adipose depots is lacking. Most European guidelines on physical activity advise to perform at least 150 minutes per week of moderate to vigorous physical activity and to limit sedentary time [9], as sedentary behaviour has been associated with increased risk of type 2 diabetes, cardiovascular disease, and cancer, even after adjustment for physical activity [10]. Obviously, less time spent sedentary implies more time spent in other activities. For assessment of the association of one particular type of activity with a certain outcome, it is therefore important to take into account with which activity this is replaced, e.g., whether sedentary time is replaced with the same time spent while physically active. Although most studies that applied such isotemporal substitution analysis to examine replacement of sedentary time by time spent on other activities in relation to body fat used objectively assessed physical activity, most used surrogate outcomes for adiposity such as body mass index or waist circumference [11] instead of direct measures of adipose tissue. Only one study on isotemporal substitution of habitual activities used direct measurements of both physical activity and visceral fat, and it was reported that replacing sedentary time with time spent on moderate physical activity was associated with a reduction in visceral fat [12]. However, liver fat was not assessed in this study and adjustment for total body fat was not performed, which may attenuate associations.

Knowledge on how different types of daily activities and their mutual substitutions are associated with different adipose depots, such as total body fat, visceral and liver fat, helps elucidating the underlying mechanism of how sedentary time or physical inactivity can lead to multiple adverse health outcomes. This may lead to more specified guidelines on sedentary time and physical activity. We therefore aimed to study the association between substitution of sedentary time with other daily activities and total body fat, visceral fat and liver fat in a population-based cohort of middle-aged participants.

METHODS

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study designed to investigate pathways that lead to obesity-related diseases. The NEO study started in 2008 and includes 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. The study design and data collection are described in detail elsewhere [13]. Men and women living in the greater area of Leiden (in the West of the Netherlands) were invited to participate if they were aged between 45 and 65 years and had a self-reported BMI of 27 kg/m^2 or higher. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study. All participants gave their written informed consent.

Participants were invited to a baseline visit at NEO study center of the LUMC after an overnight fast. Prior to this study visit, participants collected their urine over 24 hours and completed a general questionnaire at home to report demographic, lifestyle and clinical information. The participants were asked to bring all medication they were using to the study visit. At the study center, the participants completed a screening form, asking about anything that might create a health risk or interfere with magnetic resonance imaging (MRI) (most notably metallic devices, claustrophobia, or a body circumference of more than 1.70 m). Of the participants who were eligible for MRI, approximately 35% were randomly selected to undergo direct assessment of abdominal fat. Another random subsample (n=955) received the Actiheart device (Actiheart, CamNtech Ltd, UK) to carry for four days to objectively assess daily levels of physical activity.

The present study is a cross-sectional analysis of the baseline measurements of the NEO study. We excluded participants with missing data on objectively assessed physical activity, body fat measurements or potential confounding factors.

Objective assessment of daily activities

Daily levels of physical activity were objectively assessed the NEO study participants (n=955) who carried the Actiheart device, a uniaxial activity monitor capable of measuring acceleration and heart rate (Actiheart, CamNtech Ltd, UK). The device weighs less than 8g and is worn directly onto the skin. Two standard ECG electrodes (H98SG, Tyco Healthcare, Germany) were placed at the level of the second intercostal space; one on the sternum and one 10 cm to the left of the first electrode. Participants were instructed to wear the monitor continuously for four consecutive days and nights, except when showering, bathing or swimming, and to carry on with all normal activities during this time. The monitor was set-up to record at 15 s epochs.

A Gaussian process regression method was applied to the heart rate data to handle potential measurement noise [14]. Using a branched equation algorithm the acceleration and heart rate information was summarised into calibrated estimates of physical activity energy expenditure (PAEE) and time spent at different activity intensities expressed as metabolic equivalents of task (MET) [15, 16]. When summarising the data we accounted for non-wear time and any potential diurnal imbalance of wear time by weighting all hours of the day equally in the summation [17]. In a subgroup of 132 participants who were equipped with an Actiheart monitor, an 8-min ramped step test was performed to calibrate the individual heart rate response to activity intensity. In addition, a group calibration equation was applied to the results of the other participants, which was derived from the valid step tests in this population [18].

Sedentary time was defined as time spent in activities with an intensity ≤1.5 MET, excluding sleep time. Sleep time was assumed as the time between 23:00h and 07:30h on weekdays and between 23:30h and 07:30h on weekend days unless Actiheart detected activity took place. Light intensity physical activity (LPA) was defined as any activity during wear time with an intensity >1.5 and ≤3 MET. Moderate-to-vigorous physical activity (MVPA) was defined as any activity >3 MET [19]. Participants with a valid wear time <24 hours were excluded from the analyses. No minimum bout duration was set for the activity intensity categories.

Assessment of body fat

Body weight and percent body fat were assessed by a Tanita bio impedance balance (TBF-310, Tanita International Division, UK) without the participant wearing shoes and one kilogram was subtracted from the body weight. BMI was calculated by dividing the weight in kilograms by the height in meters squared. In a random subgroup without contraindications, imaging was performed on a 1.5 Tesla MR system (Philips Medical Systems, Best, the Netherlands). Visceral adipose tissue (VAT) was quantified by a turbo spin echo imaging protocol using MRI. At the level of the fifth lumbar vertebra, three transverse images each with a slice thickness of 10 mm were obtained during a breath-hold. Visceral fat area was converted from the number of pixels to centimeters squared using in-house-developed software (MASS, Medis, Leiden, the Netherlands) and the average of three slices was used in the analyses [13]. Hepatic triglyceride content (HTGC) was quantified by proton-MR spectroscopy (^{1}H-MRS) of the liver [20]. An 8 ml voxel was

positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Sixty-four averages were collected with water suppression. Spectra were obtained with an echo time of 26 ms and a repetition time of 3,000 ms. Data points (1,024) were collected using a 1,000 Hz spectral line. Without changing any parameters, spectra without water suppression, with a repetition time of 10 s, and with four averages were obtained as an internal reference. ^{1}H-MRS data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium), as described previously [21]. Hepatic triglyceride content relative to water was calculated as the sum of signal amplitudes of methyl and methylene divided by the signal amplitude of water and then multiplied by 100.

Confounding factors

On the questionnaire, participants reported ethnicity by self-identification in eight categories which we grouped into white (reference) and other. Tobacco smoking was reported in the three categories current, former, and never smoking (reference). Highest level of education was reported in 10 categories according to the Dutch education system and grouped into high (including higher vocational school, university, and post-graduate education) versus low education (reference). Participants reported their medical history of diabetes and cardiovascular diseases. Pre-existing cardiovascular disease was defined as myocardial infarction, angina, congestive heart failure, stroke, or peripheral vascular disease. Habitual dietary intake of all participants was estimated using a semi-quantitative self-administered 125-item food frequency questionnaire (FFQ) [22, 23]. Based on these variables, an adapted version of the Dutch Healthy Diet Index (DHD-index) 2015 was calculated which consisted of 13 components (vegetables, fruit, wholegrain products, legumes, unsalted nuts, dairy, fish, tea, liquid to solid fat ratio, red meat, processed meat, sweetened beverages and alcohol). The DHD-index ranges between 0 and 130, in which a higher score reflects better adherence to the Dutch Guidelines for a Healthy Diet of 2015 [24].

Statistical analysis

In the NEO study there is an oversampling of persons with a BMI of 27 kg/m^2 or higher. To correctly represent associations in the general population [25], adjustments for the oversampling of individuals with a BMI ≥ 27 kg/m^2 were made. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality [26], whose BMI distribution was similar to the BMI distribution of the general Dutch population [27]. All results were based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI ≥ 27 kg/m^2. Because of the weighted analyses, percentages and proportions are given instead of numbers of participants. Other baseline characteristics are expressed as mean with standard deviation.

We performed linear regression analyses and fitted several models. First, we examined the association between 30 minutes of daily activities (i.e. sedentary time, light, and moderate to vigorous physical activity) and total body fat, visceral fat and liver fat in a crude model. Second, a multivariable model was applied that was adjusted for sex, age, ethnicity, education, DHD-index, and smoking. This model describes the association between 30 minutes/day of each of the daily activities on top of the regular activity pattern for each outcome. Third, we performed an isotemporal substitution approach in our final model including waking time and excluding sedentary time. Consequently, the regression coefficients of each activity represent the estimated difference in measure of body fat associated with replacing 30 minutes of sedentary time with 30 minutes spent on this type of activity. This isotemporal substitution model on visceral fat and liver fat was additionally adjusted for total body fat to study whether physical activity is associated with visceral fat and liver fat beyond effects via total body fat. Regression coefficients of total body fat represent an absolute difference in TBF in % per 30 minutes of a certain activity, and those of VAT an absolute difference in VAT in cm^2 per 30 minutes of a certain activity per day.

Due to a skewed distribution of HTGC, we used the natural logarithm of this variable in the analyses. For interpretation of the results, we back-transformed the regression coefficients of HTGC towards a ratio with 95% confidence interval, which indicates the relative excess liver fat content for each 30 minutes per day spent additionally on a certain activity. This ratio, for example a ratio of 1.2, can be interpreted as each 30 minutes per day of sedentary time replaced with similar time spent performing moderate to vigorous physical activity being associated with a 1.2-fold HTGC. This would reflect an increase in liver fat from, for example, 5% to 6% .

Data was analyzed using STATA v.14 (StataCorp LP, College Station, TX, USA).

RESULTS

Physical activity was objectively assessed in 955 participants, of whom 932 had a valid measurement. The group of participants with a physical activity assessment was similar in sex distribution, age, and BMI to the group without an objective assessment of physical activity, but were slightly less likely to have a history of CVD (5.6% versus

6.1%). We excluded participants with fewer than 24 hours of measurement (n=39) or for whom daily activities could not be estimated (n=32). For the analyses on total body fat, we excluded participants with missing data on smoking status (n=1), education (n=7) and ethnicity (n=1), leaving a total of 852 participants.

These participants (41% men) had a mean (SD) age of 56 (6) years and BMI of 26 kg/m^2 (4). Participants had a mean (SD) valid Actiheart wear time of 83 (12) hours and 80% of participants had more than 72 hours of valid wear time. Most waking time was spent sedentary, and least time performing vigorous physical activity (**Figure 1**). The baseline characteristics of the total population for the analyses on total body fat stratified by tertiles of moderate to vigorous physical activity are shown in **Table 1**. Participants in the highest tertile of activity were slightly younger, more often men, more often non-smokers, had a lower BMI and spent less time in sedentary time than participants in the lowest tertile of MVPA.

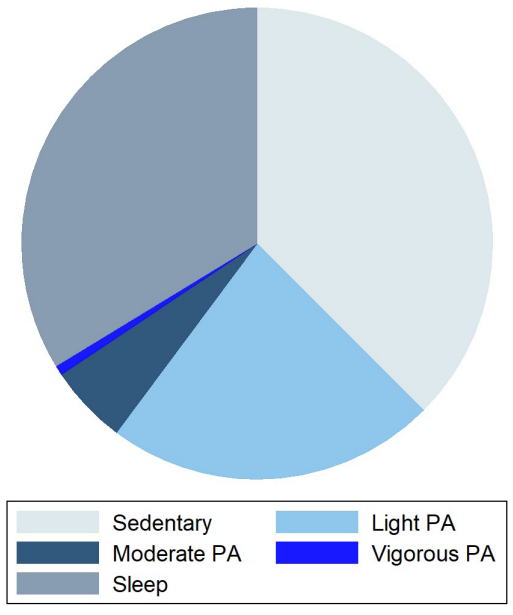

Figure 1. Distribution of daily activities during an average 24-hour period in participants with a physical activity and total body fat measurement in the Netherlands Epidemiology of Obesity study (N=852)

Table 1. Characteristics of participants of the Netherlands Epidemiology of Obesity study , men and women between 45 and 65 years of age with objectively assessed physical activity stratified by tertiles of moderate to vigorous physical activity

	Tertiles of moderate to vigorous physical activity (min/d)		
	T1 (0.2-44.8)	T2 (45.4-85.5)	T3 (85.6-394.8)
Age (y)	57 (6)	56 (6)	55 (6)
Sex (% men)	37	32	53
Ethnicity (% white)	95	95	97
Education level (% high)[1]	33	45	42
BMI (kg/m^2)	27.4	26.2	25.4
Tobacco smoking (% current)	22	15	12
Dutch Healthy Diet Index	69.0 (16.1)	72.0 (13.6)	71.7 (13.7)
HDL-Cholesterol (mmol/l)	1.5 (0.5)	1.6 (0.5)	1.6 (0.4)
Triglycerides (mmol/l)	1.4 (0.9)	1.2 (0.7)	1.1 (0.7)
CVD (%)	12	2	6
Sedentary time while awake (min/day)	523 (76)	445 (70)	362 (80)
LPA (min/day)	256 (93)	325 (92)	369 (98)
MPA (min/day)	27 (13)	61 (12)	120 (47)
VPA (min/day)	1 (3)	4 (6)	14 (16)
Total body fat (%)			
Men	25.6 (7.2)	26.0 (5.9)	23.7 (5.5)
Women	38.3 (7.8)	37.2 (5.8)	34.7 (5.5)
Visceral fat (cm^2)[2]			
Men	118.9 (73.8)	122.4 (62.2)	103.6 (53.1)
Women	88.6 (52.6)	73.3 (35.0)	46.2 (22.7)
Liver fat (%)[3]			
Men	7.8 (7.7)	10.3 (15.1)	6.7 (8.2)
Women	5.9 (9.6)	5.2 (8.3)	1.9 (2.5)

From this population, 294 participants had a measurement of visceral fat by MRI. These 294 participants were similar in age (mean 56, SD 6) to those without an MRI of the abdomen (mean 56, SD 6) and CVD history (both 6%), but were more often men (45% versus 39%) and had a slightly lower BMI (25.7 kg/m^2 versus 26.5 kg/m^2).

Lastly, for the analyses on liver fat, we additionally excluded participants without ^{1}H-MRS measurement (n=45) and those who consumed 40 grams of alcohol or more (4 standard glasses) per day (n=21), leaving a total of 228 participants.

Sedentary time and physical activity in relation to total body fat

After adjustment for confounding, 30 minutes per day of sedentary time was associated with 0.27% more total body fat (95% CI 0.11, 0.43), whereas 30 minutes per day of light physical activity (-0.25%, 95% CI -0.49, -0.02) and moderate to vigorous physical activity (-0.61%, 95% CI -0.97, -0.25) were associated with less total body fat. In the isotemporal

substitution model, replacing 30 minutes of sedentary time per day with 30 minutes of moderate to vigorous physical activity was associated with less total body fat (-0.51%, 95% CI -0.94, -0.07), whereas light physical activity (-0.17%, 95% CI -0.45, 0.11) was not **(Table 2)**.

Table 2. Associations of daily activities per 30 minutes with total body fat (%) in participants with a measurement of total body fat by bioimpedance analysis and physical activity by Actiheart

	Crude	Multivariable[1]	Substitution model[2]
Per 30 min/day	**% TBF (95% CI)**	**% TBF (95% CI)**	**% TBF (95% CI)**
Sedentary time	0.15 (-0.06; 0.36)	0.27 (0.11; 0.43)	*substituted*
Light physical activity	0.04 (-0.22; 0.30)	-0.25 (-0.49; -0.02)	-0.17 (-0.45; 0.11)
Moderate to vigorous physical activity	-1.15 (-1.58; -0.72)	-0.61 (-0.98; -0.25)	-0.51 (-0.94; -0.07)

Results are based on analyses weighted toward the BMI distribution of the general population (n=852).
[1]Adjusted for sex, age, ethnicity, education, DHD-index and smoking
[2]Additionally adjusted for total time awake, and for all other daily activities but not for sedentary time. Coefficients represent the association between substitution of 30 minutes sedentary time with 30 minutes of either light or moderate to vigorous physical activity and total body fat (%).

Sedentary time and physical activity in relation to visceral fat

In adjustment analyses, 30 minutes per day of sedentary time was associated with 2.0 cm² more visceral fat (95% CI 0.1, 4.9), whereas 30 minutes per day of moderate to vigorous physical activity was associated with 6.7 cm² less (95% CI -10.3, -3.1) visceral fat. Light physical activity was not associated with visceral fat (-1.0 cm², 95% CI -3.4, 1.3). In the isotemporal substitution model, replacing 30 minutes per day of sedentary time with 30 minutes per day of moderate to vigorous physical activity was associated with 7.2 cm² less (95% CI -10.7, -3.6) visceral fat, whereas replacement with light physical activity was not associated with visceral fat (0.4 cm², 95% CI -2.1, 2.9). This association attenuated after additional adjustment for total body fat (**Table 3**).

Sedentary time and physical activity in relation to liver fat

In adjusted analyses, 30 minutes per day of sedentary time was associated with more liver fat in adjusted analyses (1.05-fold, 95% CI 1.01, 1.10) whereas 30 minutes per day of moderate to vigorous physical activity was associated with less liver fat (0.88-folds, 95% CI 0.81, 0.96). Light physical activity seemed not or only marginally associated with liver fat (0.96-fold, 95% CI 0.91, 1.02). In the isotemporal substitution model, replacing 30 minutes per day of sedentary time with 30 minutes per day of moderate to vigorous physical activity was associated with less liver fat (0.89-fold, 95% CI 0.82, 0.97), whereas replacement with light physical activity showed little association with liver fat (0.98-fold, 95% CI 0.92, 1.04). This association attenuated after additional adjustment for total body fat (**Table 4**).

Table 3. Associations of daily activities per 30 minutes with visceral fat (cm²) in participants with a direct measurement of visceral fat by MRI and physical activity by Actiheart

	Crude	Multivariable[1]	Substitution model[2]	Substitution model[3]
Per 30 min/day	**cm² VAT (95% CI)**	**cm² VAT (95% CI)**	**cm² VAT (95% CI)**	**cm² VAT (95% CI)**
Sedentary time	3.7 (2.0; 5.4)	2.0 (0.1; 3.9)	*substituted*	*substituted*
Light physical activity	-3.2 (-5.1; -1.2)	-1.0 (-3.4; 1.3)	0.4 (-2.1; 2.9)	0.4 (-1.6; 2.4)
Moderate to vigorous physical activity	-8.2 (-11.8; -4.6)	-6.7 (-10.3; -3.1)	-7.2 (-10.7; -3.6)	-0.6 (-3.9; 2.7)

Results are based on analyses weighted toward the BMI distribution of the general population (n=294).
[1]Adjusted for sex, age, ethnicity, education, DHD-index and smoking
[2]Additionally adjusted for all other daily activities
[2]Additionally adjusted for total time awake, and for all other daily activities but not for sedentary time. Coefficients represent the association between substitution of 30 minutes sedentary time with 30 minutes of either light or moderate to vigorous physical activity and visceral fat (cm²).
[3]Additionally adjusted for total body fat

Table 4. Associations of daily activities per 30 minutes with hepatic triglyceride content (%) in participants with a direct assessment of hepatic triglyceride content by ¹H-MRS and physical activity by Actiheart

	Crude	Multivariable[1]	Substitution model[2]	Substitution model[3]
Per 30 min/day	**Relative change in HTGC (95% CI)**	**Relative change in HTGC (95% CI)**	**Relative change in HTGC (95% CI)**	**Relative change in HTGC (95% CI)**
Sedentary time	1.08 (1.03; 1.12)	1.05 (1.01; 1.10)	*Substituted*	*Substituted*
Light physical activity	0.94 (0.89; 0.99)	0.96 (0.91; 1.02)	0.98 (0.92; 1.04)	0.98 (0.94; 1.03)
Moderate to vigorous physical activity	0.87 (0.80; 0.94)	0.88 (0.81; 0.96)	0.89 (0.82; 0.97)	1.00 (0.92; 1.09)

Results are based on analyses weighted toward the BMI distribution of the general population (n=228).
[1]Adjusted for sex, age, ethnicity, education, DHD-index and smoking
[2]Additionally adjusted for all other daily activities
[2]Additionally adjusted for total time awake, and for all other daily activities but not for sedentary time. Coefficients represent the association between substitution of 30 minutes sedentary time with 30 minutes of either light or moderate to vigorous physical activity and hepatic triglyceride content.
[3]Additionally adjusted for total body fat

DISCUSSION

In this population-based cohort study of middle-aged men and women, more sedentary time was associated with more total body fat, visceral fat and liver fat, whereas moderate to vigorous physical activity was associated with less total body fat, visceral fat and liver fat. Replacing 30 minutes of sedentary time with moderate to vigorous physical activity was associated with reduced total body fat, visceral fat and liver fat. Replacing 30 minutes of sedentary time with light physical activity was not associated with total body fat, visceral fat or liver fat. These associations with visceral fat and liver fat attenuated after additional adjustment for total body fat.

To our knowledge, there is only one previous study that reported isotemporal substitution analysis with objectively measured physical activity in combination with direct measures of adiposity [12]. There, isotemporal substitution of 1 hour per day of sedentary and light intensity physical activity with other types of physical activity was associated with less visceral fat [12], which is in line with our findings. However, in this study liver fat was not assessed and analyses were adjusted for BMI rather than for objectively measured total body fat. Our study adds to this that we also assessed associations with liver fat, and were able to additionally adjust for total body fat. Our results show that replacing sedentary time with moderate to vigorous physical activity is associated with less total body fat, visceral fat and liver fat, whereas replacing sedentary time with light physical activity has no or minimal effect. The intensity of light physical activity appears to be too low . A recent systematic literature review has shown that multiple studies have investigated physical activity in relation to adiposity by means of isotemporal substitution analyses. However, most use body mass index or waist circumference as an outcome [11]. This review described that replacing 30 min/day of sedentary time with moderate to vigorous physical activity resulted in a decreased waist circumference, body mass index and body fat percentage in healthy adult populations [11]. Moreover, reallocating sedentary time to light or moderate to vigorous physical activity was associated with multiple favourable cardiometabolic biomarkers, such as insulin sensitivity [11], which may possibly be due to the associated lower body fat percentage we observed in our study.

In our study, we observed that replacing sedentary time with moderate to vigorous physical activity was associated with less total body fat, visceral fat and liver fat. However, the associations with visceral fat and liver fat attenuated when total body fat was included in the model. This attenuation for associations between physical activity and visceral fat and liver fat was also observed in other studies that included BMI into the model [12, 28]. It therefore seems that there is no extra effect on visceral fat beyond effects via total body fat.

Several previous studies reported associations between time spent in sedentary time and the risk of non-alcoholic fatty liver [29-32], although in only few studies sedentary time was measured objectively [32]. In one study researchers aimed to assess whether objectively measured levels of sedentary time and physical activity correlated with levels of directly assessed visceral fat and liver fat in 82 overweight or obese adults, but found no relationship between physical activity and liver fat and a weak positive association between time spent in moderate physical activity and visceral fat [28]. Contrastively, a twin study of both monozygotic and dizygotic twins who were discordant for physical activity based on questionnaires during a follow-up of more than 30 years showed that habitual physical activity potentially prevents accumulation of visceral and ectopic fat [33]. These findings are consistent with our results, which show that sedentary time was positively associated with visceral fat and liver fat, and moderate to vigorous activity negatively. Prospective studies with objective assessment of physical activity and sedentary time are needed to confirm these associations.

Besides abdominal adiposity, sedentary time has also been associated with other adverse metabolic consequences. Recent studies have shown that a high amount of time spent in sedentary time is associated with multiple adverse health outcomes, among which type 2 diabetes [34]. This association was also present in fit individuals [35]. Furthermore, time spent sedentary predicts higher levels of fasting insulin independent of time spent performing moderate to vigorous physical activity [36], indicating that the detrimental effects of sedentary time are not merely due to a lack of sufficient physical activity. Large meta-analyses have also shown that high sedentary time was associated with all-cause mortality [37], type 2 diabetes incidence, and cardiovascular disease and cancer risk, even after adjustment for physical activity [10]. However, the underlying biological pathways via which sedentary time may lead to disease remain largely unknown, although it has been suggested that replacing sitting with standing and LPA improves insulin sensitivity and decreases plasma triglycerides, thereby leading to a decrease in intrahepatic triglyceride storage [38, 39]. In our study, we observed that replacing standing with light or moderate to vigorous physical activity was not associated with liver fat after adjustment for total body fat, suggesting that the association is mainly driven by overall adiposity.

Important strengths of this study are the objective assessment of physical activity and sedentary time using an Actiheart monitor that combines a heart rate monitor and an accelerometer into a single device, which has been shown to classify physical activity more accurately than individual measures [40-42], and the direct assessment of visceral adipose tissue and hepatic triglyceride using MRI and ^{1}H-MRS. Another strength is that we applied isotemporal substitution analysis to be able to investigate replacement of sedentary time with light and moderate to vigorous physical activity in relation to measures of body fat. Finally, the extensive phenotyping allowed detailed adjustment for confounding factors.

A few limitations should also be discussed. Because both the physical activity measurements and the body fat measurements were performed in a random subset of the participants of the Netherlands Epidemiology of Obesity study, the number of participants in the analyses on visceral fat and liver fat were relatively small. Our results apply only to people without contraindications for MRI and with a valid Actiheart

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measurement. However, previous studies that reported on the associations between objectively measured daily activities and visceral fat and liver fat as measured by MRI and MRS included smaller sample sizes (N<100)[28,32]. Additionally, even though the Actiheart combines accelerometry with heart rate monitoring, which provides valid estimates of physical activity intensity, defining sedentary time may be less valid as information on posture is lacking. In addition, sleep and wake times were not available and therefore we used general times during which we assumed participants were asleep. This may have led to over- or underestimation of sedentary time. Furthermore, our population consisted mainly of Caucasian participants, and results need to be confirmed in other ethnic groups. Lastly, inherent to the observational cross-sectional study design we cannot exclude residual confounding and reverse causation, as people with more body fat may be less physically active because of their higher weight. Reallocations of sedentary time to light or moderate to vigorous physical activity are model-based and actual changes might not represent causal relationships. Clinical trials should therefore confirm whether actual reallocation of sedentary time with other activities leads to decreases in visceral fat and liver fat content.

To conclude, in this population-based study of middle-aged men and women, sedentary time was associated with more total body fat, visceral fat, and liver fat. Replacing 30 minutes per day with moderate to vigorous physical activity, but not light physical activity, was associated with less total body fat, visceral fat and liver fat. The associations for visceral fat and liver fat attenuated after additional adjustment for total body fat, suggesting that there is no extra effect on visceral fat and liver fat beyond effects via total body fat. This study provides knowledge on how a reduction of sedentary time by replacing it with moderate to vigorous physical activity is negatively associated with multiple adipose tissue depots, which is important for the prevention of overall and abdominal obesity, and ultimately cardiometabolic diseases.

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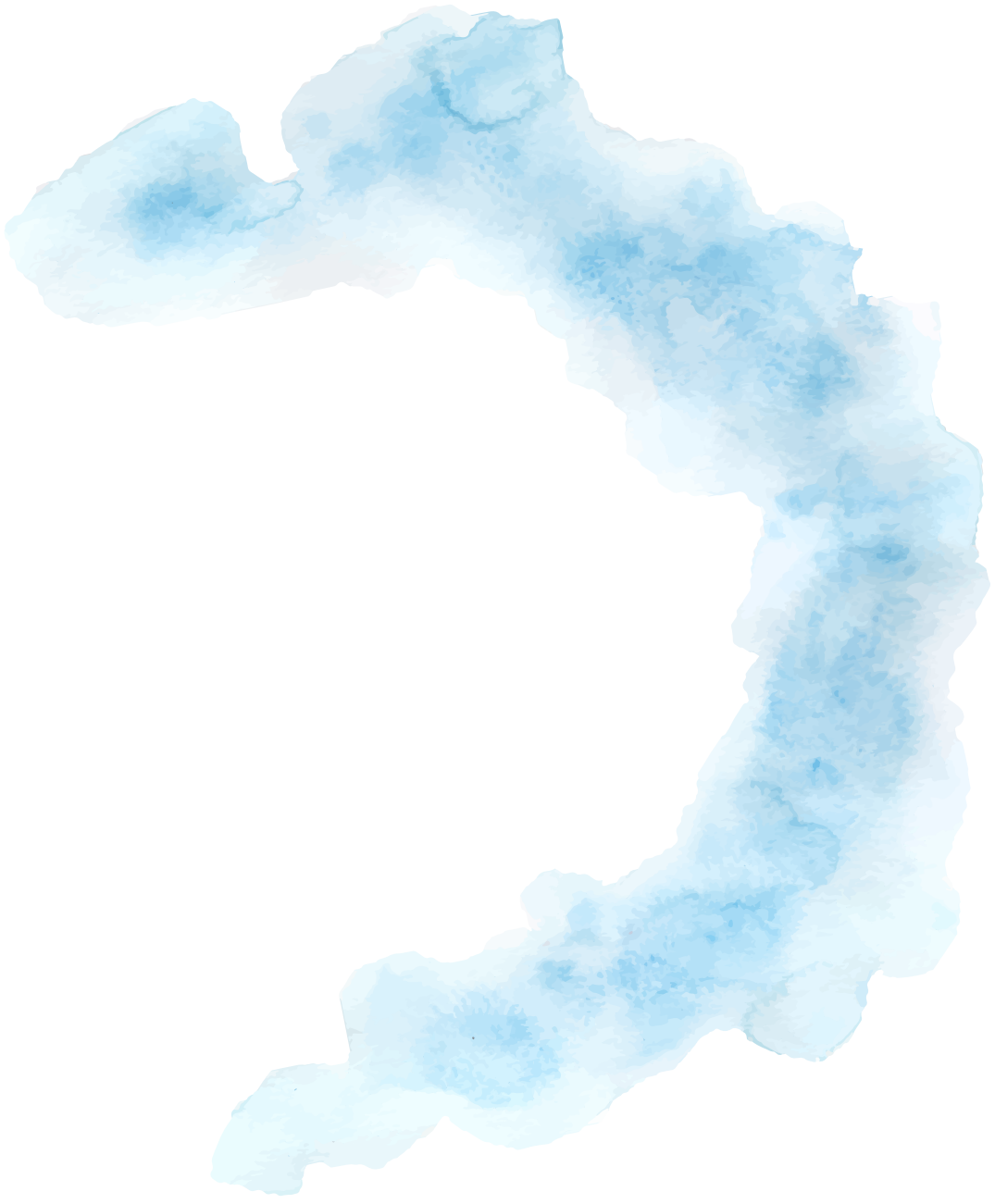

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General discussion and summary

The main aim of this thesis was to study the role of dietary habits and physical activity in the accumulation of visceral fat and liver fat. The majority of the studies described in this thesis were performed in the Netherlands Epidemiology of Obesity study, in which middle-aged participants underwent deep phenotyping, including measures of visceral adipose tissue by magnetic resonance imaging (MRI) and hepatic triglyceride content by magnetic resonance spectroscopy (MRS). We used the baseline measurement of NEO participants to investigate the association between dietary habits or physical activity patterns and visceral fat volume and liver fat content. In this general discussion we summarize our main findings, discuss their interpretation and address implications for future research.

SUMMARY OF MAIN FINDINGS

In **Chapter 2** we show the results of our systematic review and meta-analysis of randomized controlled trials and provide a summary of the evidence on the effect of dietary macronutrient composition on liver fat content as assessed by ^{1}H-MRS, MRI, CT or liver biopsy in adults. We concluded that a diet high in saturated fat leads to more liver fat accumulation than a diet high in unsaturated fat. When a diet high in protein is compared with a diet high in carbohydrates, replacing carbohydrates with proteins decreases liver fat content. Exchanging carbohydrates for dietary fats did not lead to changes in liver fat, although results of different trials were in conflict. Since only a limited number of eligible trials could be included in these meta-analyses, we have identified an essential gap in knowledge on the effect of dietary macronutrient composition on liver fat content.

In **Chapter 3** we describe the relation between dietary intake of multiple food groups and measures of visceral adipose tissue and hepatic triglyceride content. Consumption of fruit and vegetables and plant-based fats and oils was associated with less visceral fat, whereas consumption of sweet snacks was associated with more liver fat in the total study population. Patterns of were similar in men and women. Associations were partly explained by total body fat, as they attenuated after additional adjustment for total body fat, but remained present.

In **Chapter 4** we show that a higher score on the Dutch Healthy Diet Index, which reflects a better adherence to the 2015 Dutch dietary guidelines, was associated with less total body fat, less visceral fat and less liver fat. The associations with visceral fat and liver fat remained present after adjustment for total body fat, indicating that the associations are indeed specific for visceral and liver fat rather than merely representing associations with overall adiposity. We observed that associations were not driven by one component in particular, but rather that all components seemed important.

Besides food items or food groups, excessive alcohol consumption is a well-known risk factor for liver fattening and liver disease. However, it was unknown whether moderate alcohol consumption is also associated with liver fat content, and energy-containing non-alcoholic beverages might also contribute to liver fat accumulation. In **Chapter 5** we report that each additional serving of alcoholic beverages per day was indeed associated with more liver fat. Light and moderate consumption were not associated with liver fat. Nevertheless, replacing one alcoholic serving with one non-alcoholic serving was associated with less liver fat. Isocaloric replacement (5 En%) of alcoholic beverages with sugar sweetened beverages was equally associated with liver fat, whereas substitution of 5 En% alcohol with 5 En% milk was associated with less liver fat.

In addition to dietary habits, physical activity is an important cornerstone in the prevention of obesity. Not only exercise, but also habitual, unstructured activity is essential. In **Chapter 6** we studied different objectively measured activity levels of physical activity (sedentary behaviour, light, moderate and vigorous activity) in relation to total body fat, visceral fat and liver fat. We observed that sedentary time was associated with more total body fat, visceral fat and liver fat, whereas moderate to vigorous physical activity was associated with less total body fat, visceral fat and liver fat. Replacing 30 minutes of sedentary time per day with moderate to vigorous physical activity was associated with less total body fat, visceral fat and liver fat. These associations with visceral fat and liver fat disappeared after additional adjustment for total body fat. It therefore seems that there is no extra effect on visceral fat and liver fat beyond effects via total body fat.

METHODOLOGICAL CONSIDERATIONS

Before we can causally interpret the findings described in this thesis, several methodological considerations need to be discussed. For **Chapters 3** to **6**, baseline measurements of the Netherlands Epidemiology of Obesity study were used. This study has been set up in 2008 to investigate pathways that lead to obesity-related diseases. A strength of this population based study is that the large study population has been extensively phenotyped, which allowed us to adjust for multiple confounding factors and to study possible sex differences. Moreover, physical activity has been measured

objectively, and direct imaging of visceral fat and liver fat has been performed. A limitation, however, is the observational and cross-sectional nature of the analyses performed on the baseline measurements. Residual confounding due to unmeasured lifestyle factors might be present despite our efforts to minimize confounding as much as possible. Moreover, the cross-sectional design and possible reverse causation precludes causal inference. In the following paragraphs we will discuss these and other limitations regarding the study design and data collection from the NEO study, and how we attempted to minimize the potential bias and confounding that might have resulted from those limitations.

Internal validity: bias and confounding by lifestyle

During the past year, a substantial amount of criticism on nutritional epidemiology has been expressed. Whereas results from nutritional epidemiological studies are often presented as causal ones, most risks or benefits associated with dietary habits are said to mainly reflect the magnitude of different types of bias and residual confounding, since most of the studies are of an observational nature [1]. Furthermore, most nutritional variables are correlated with each other and therefore associations between one specific dietary variable and health outcomes might not be specific, but rather represent associations between other dietary variables and health outcomes [1]. By combining multiple food items into food groups and also studying dietary quality as measured by the Dutch Healthy Diet Index, we have aimed to minimize this problem. Furthermore, when studying food groups, we also adjusted for a marker of a healthy diet in order to adjust for potential correlations between the food group under study and overall diet.

The study of an association between consumption of foods or food groups in relation to visceral fat or liver fat poses problems. In free-living populations, consumption of most nutrients and thereby food items is positively correlated with total caloric intake [2]. Increased consumption of a certain food item means that the total caloric intake also increases due to the caloric contribution of the macronutrients from that specific food item. Moreover, large persons, or those who are more physically active, on average consume more of everything and therefore even food items with very low to no caloric value are correlated with total energy intake [2]. If caloric intake is also associated with the outcome, for example total body fat, this may overestimate the association between the food item and the outcome. In order to control for this, an increase in consumption of a certain food item must be accompanied by a decrease in consumption of another food item to keep total caloric intake similar. This can be modelled using substitution analyses, by defining the contrast of which food item is to be replaced by the food item under study.

From origin, substitution analyses have been used to study and compare the effects of different macronutrients on multiple health outcomes in an isocaloric manner [3]. This statistical method has become increasingly used throughout the entire field of nutritional epidemiology, and also in the field of physical activity [4]. It entails a statistical technique to mimic a trial in which the dietary composition is altered, without changing the total caloric intake. In this thesis, we used substitution analyses to study replacement of dairy with other food groups, replacement of alcoholic by non-alcoholic beverages, and replacement of sedentary time with time spent on other types of physical activity. However, compared with the study of macronutrients, that of substitution of food groups or items leads to additional methodological considerations [5]. Firstly, the results are dependent on the reference group that is chosen. Although theoretically any two food groups could be chosen for substitution analysis, results will become meaningless without cautious considerations [5]. For instance, the replacement of a food group with detrimental health effects to a food group with even worse health effects will yield an association that appears beneficial, and results could be misleading. Therefore, it is advised that food items or groups used for substitution analysis should be part of a well-defined category and commonly consumed within the population under study. They should also be relevant for replacement, with desirably a fair inverse correlation [5]. Furthermore, substitution analyses with food items or food groups may amplify confounding [5]. In contrast to nutrients, which are mainly consumed to meet the body's energy requirements, consumption of food items or groups is strongly influenced by health-related behaviours. For example when meat is substituted with fish: consumption of meat may be more associated with unhealthy behaviour, whereas fish consumption with healthy behaviours. An analysis in which meat is substituted for fish may therefore not only represent an exchange of food products, but also indirectly an exchange of behaviours, and the net effect of the substitution is overestimated. This also holds true when using substitution analysis for physical activity. Adequate adjustment for lifestyle factors is therefore of great importance. Nonetheless, results of the substitution analyses described in this thesis may be overestimated despite our efforts to minimize confounding and need to be interpreted cautiously. It should be noted that even if we interpret the substitution analysis causally, which means that if individuals had replaced item A with item B, they would have had less, say, liver fat, this does not mean that substitution would lead to a reduction of liver fat.

Measurement error

Most large epidemiological cohort studies rely on self-reported body mass index or waist circumference as an indication of adiposity. In the NEO study, however, body weight and percent body fat were assessed by the Tanita foot-to-foot bio impedance balance system

(TBF-310, Tanita International Division, UK). Although it has been suggested that foot-to-foot BIA might give an overestimation of the amount of fat mass [6], a strong correlation (r = 0.84) has been shown between foot-to-foot and hand-to-foot BIA with regard to total body fat percentages [7]. Furthermore, a strong correlation (r = 0.89) was also found in a study comparing resistance measurements provided by foot-to-foot BIA with measurements from dual-energy X-ray absorptiometry and underwater weighing [8].

Additionally, a unique feature of the NEO study is that abdominal adipose tissue was assessed using imaging techniques in a random subsample of the total NEO population without contra-indications to MRI [9]. In total, abdominal adiposity was assessed in 2,580 participants using a turbo spin echo imaging protocol. At the level of the 5th lumbar vertebra 3 transverse images each with a slice thickness of 10 mm were obtained during a breath-hold [10]. Hepatic triglyceride content was assessed using proton MRS of the liver in the same subset of participants. However, the number of participants with technical failures for liver fat measurement was relatively high because only a limited time slot was available per participant and therefore it was not possible check the spectra during the measurement and repeat the measurement when technical failures were present. However, the failure rate was not related to age, sex, waist circumference, BMI, total body fat or amount of visceral fat [11]. Based on this information, we can conclude that the group in which the liver fat assessment was successful was a completely random subgroup of the total group of participants who underwent the proton MRS.

In **Chapters 3, 4 and 5** we used a food frequency questionnaire to estimate dietary habits. Although such questionnaires are suited for large epidemiological studies, they are notorious for their risk of bias, and estimates of intake may be subject to substantial error, which in turn may affect the interpretation of epidemiological studies [12-14]. Social desirability in the way respondents fill in items can result in an overestimation of intake of healthy foods, and an underestimation of intake of unhealthy foods [15]. Especially obese participants may underreport their total energy intake [16] and fatty foods or foods rich in carbohydrates [17], but no major differences were found between men and women [17]. Kipnis and colleagues have described two types of dietary measurement error that can occur when a food frequency questionnaire is used: intake-related bias, which represents the correlation between the error and true intake, and person-specific bias, which is independent of the true intake and reflects measurement error related to personal characteristics of the participant. The latter can be reduced considerably by energy adjustment, which is why we adjusted all our dietary models for total energy intake, but will nonetheless remain present to a certain extent. With respect to the three studies in this thesis using a food frequency questionnaire, it means that the associations between healthy dietary habits an adiposity measures are likely underestimations of reality [18].

Reverse causation

In **Chapters 3 to 6** we performed cross-sectional analyses to study the association between diet and physical activity and body fat distribution. These analyses were based on the baseline measurements of the NEO study, meaning that the exposure and the outcome variables were measured at the same time. This poses difficulties in the interpretation of results, as we cannot always tell with certainty which came first: the exposure, or the outcome. For example, people who are told by their general practitioner that they have an increased risk of having a myocardial infarction due to their current lifestyle, are more likely to change their lifestyle and develop healthier dietary habits than the general population. However, while their diet might be healthier due to these changes, they are still more likely to suffer from a myocardial infarction than the general population. When studying the association between dietary habits and the risk of myocardial infarction, it might therefore appear as if a healthier diet is associated with an increased risk of having a myocardial infarction.

In **Chapters 3, 4, 5 and 6** this would mean that participants with a higher body fat percentage, or more visceral fat or liver fat, might have altered their dietary habits or physical activity patterns as a result of their adiposity. A higher body fat percentage or more ectopic fat has been associated with cardiovascular disease and diabetes, and worrying about this increased risk might have resulted in a change of lifestyle. If reverse causation was present in our analyses, this would have led to an underestimation of the associations, as it would appear that a healthy diet or time spent performing physical activity is not associated with less total body fat or ectopic fat. For that reason, we have repeated our analyses described in these chapters after exclusion of participants with a history of cardiovascular disease and participants who had been diagnosed with type 2 diabetes, as they might have changed their dietary habits after being diagnosed. After exclusion of these participants, results remained similar. This suggests that reverse causation did not bias our associations to a large extent.

External validity

In the NEO study, we have included 5,000 participants with a BMI of 27 kg/m^2 or higher, and a reference group of 1,671 participants irrespective of their BMI. This reference group has a normal BMI distribution, similar to and thus representative of the general Dutch population [19]. Thus, although the majority of the participants has been selected based on their body weight and therefore possibly also other weight-related factors, this

is countered by weighting our analysis towards the BMI distribution of the reference population. After weighting, results represent characteristics and associations in the general population [19].

Furthermore, visceral fat and liver fat were assessed in a random subgroup of participants without contraindications (e.g. claustrophobia, metallic devices or a body circumference of more than 1.70 meter). As a result, on average participants who underwent an MRI/MRS measurement had a slightly lower BMI and were somewhat less likely to have a history of cardiovascular disease. Results described in this thesis are therefore applicable to the general, middle-aged Dutch population without contraindications for an MRI, and may not be representative for extremely obese persons with a body circumference of more than 1.70 meter.

POTENTIAL UNDERLYING MECHANISM

After a careful review of our results and the methodological considerations that come with them, it is interesting to think about an underlying mechanism. Based on the current literature, fructose consumption might be a potential underlying mechanism that explains several of our findings in relation to liver fat as described in this thesis.

In **Chapter 2** we showed that dietary macronutrient composition and the quality of those macronutrients affects liver fat content. Whereas there was no effect of total fat with carbohydrates, the type of fat did matter: consumption of saturated fat leads to an increased liver fat content as compared with unsaturated fat, which is in line with a previous review on macronutrient composition in relation to liver fat accumulation [20]. As not all included studies in our meta-analysis provided information on which type of fat was replaced with carbohydrates, we could not assess whether replacing saturated fat with carbohydrates affects liver fat differently than replacing unsaturated fat with carbohydrates. However, the three studies that found that a low-carbohydrate high-fat diets decreases liver fat as compared with a high-carbohydrate low-fat diet all used unsaturated fat for this comparison [21, 22]. Previous research has shown that consumption of carbohydrates is a major stimulus for hepatic de novo lipogenesis and might even contribute to non-alcoholic fatty liver disease to a greater extent than dietary fat [23].

The type of carbohydrates also plays a role in the effect on liver fat. Most carbohydrates that are consumed, will enter the blood as glucose. Glucose serves as an energy source for the body and can be used by all the cells in a human body. It has a high glycaemic index, meaning it increases the blood glucose level rapidly, and stimulates production of insulin directly. Glucose is transported into cells throughout the body by the glucose transport type-4 (GLUT-4), which is an insulin-dependent transporter. A second common carbohydrate, fructose, has a low glycaemic index and does not stimulate the secretion of insulin. It is almost entirely cleared by the liver and does not signal the brain when satiety is reached, leading to an increased food consumption. Moreover, fructose is transported into cells by the glucose transporter type-5 (GLUT-5), of which most cells only have low amounts, and it is poorly absorbed by the gastrointestinal tract. The metabolism of fructose, which takes place in the liver, stimulates lipogenesis and high consumption is therefore associated with an increased hepatic de novo lipogenesis, an increased concentration and secretion of triglycerides and also with a decrease in the clearance of very low density lipoprotein triglycerides. Several studies have shown that excessive dietary fructose consumption is associated with non-alcoholic fatty liver disease [24-26]. It must be noted that a high visceral fat volume can also promote further liver fat accumulation.

CONCLUSIONS AND IMPLICATIONS

In this thesis, we aimed at answering the question to what extent dietary habits and physical activity are associated with ectopic fat. The main conclusions of this thesis are that dietary macronutrient composition is likely to play a role in the accumulation of liver fat, and that diets high in saturated fat lead to more liver fat than diets high in unsaturated fat. Increased consumption of dietary protein at the expense of carbohydrates results in less liver fat. However, our study identified an important gap in the current knowledge, and we therefore recommend that more and larger randomized controlled dietary trials should be performed in which the source and type of macronutrients should be taken into account. More specifically, trials on the exchange between dietary protein and fat should be conducted, as evidence on this comparison was completely lacking. We furthermore showed that consumption of sweet snacks was associated with increased liver fat, and consumption of fruit and vegetables with less visceral fat. Additionally, a higher score on the Dutch Healthy Diet Index was associated with less total body fat, as well as less visceral and liver fat. Consumption of alcoholic and sugar sweetened beverages was associated with more liver fat, and replacement of alcoholic beverages with milk was associated with less liver fat. Replacement of alcohol with sugar sweetened beverages was associated with an equal amount of liver fat. Replacement of 30 minutes of sedentary time per day with moderate to vigorous physical activity was associated with less total body fat, visceral fat and liver fat. In the paragraph below we

will translate our findings to implications for clinical practice and to recommendations for future research.

Clinical implications

As described in the introduction of this thesis, body fat or body mass index is not a good indicator when it comes to the risk of cardiometabolic diseases. Rather, one should focus on the amount of ectopic fat as this proves to be a more pronounced risk factor for chronic diseases. In this thesis, we have shown that dietary habits and physical activity are associated with both visceral fat and liver fat. Even after additional adjustment for total body fat most associations remained present, indicating that most exposures we studied in this thesis are specifically associated with both visceral fat and liver fat. Future research should aim to study whether these associations are indeed, as we believe, causal, and whether changes in diet and physical activity indeed lead to beneficial changes in ectopic fat accumulation. Nevertheless, our findings are promising and hint towards the importance of considering diet as a whole, instead of separate components, which is in line with the current changes in dietary guidelines throughout the world.

Based on the results described in this thesis, we are able to make some recommendations with regard to diet and physical activity. Firstly, our results are in line with the recommendations made by the Netherlands Nutrition Center, which state that consumption of sweet snacks such as chocolate or cake should be limited and consumption of fruit and vegetables should be encouraged. In line with this recommendation, people should adhere to the 2015 Dutch Dietary Guidelines for a Healthy Diet, which entails following a dietary pattern that involves more plant-based and less animal-based foods. Consumption of carbohydrates should be limited and if possible replaced with dietary protein, and saturated fat should be replaced with unsaturated fat. Consumption of alcoholic beverages should be limited. What we have added to this, is that for those who wish to limit their alcohol consumption, it is recommended to not replace this with sugar sweetened beverages. Consuming a glass of milk, tea, coffee or water instead of a glass of alcohol appears to be better. Our results are also in line with the Dutch physical activity guidelines, which state that sufficient physical activity should be performed and sedentary time should be limited. Our results indicate that, preferably, sedentary behaviour should be replaced with moderate to vigorous physical activity rather than light physical activity.

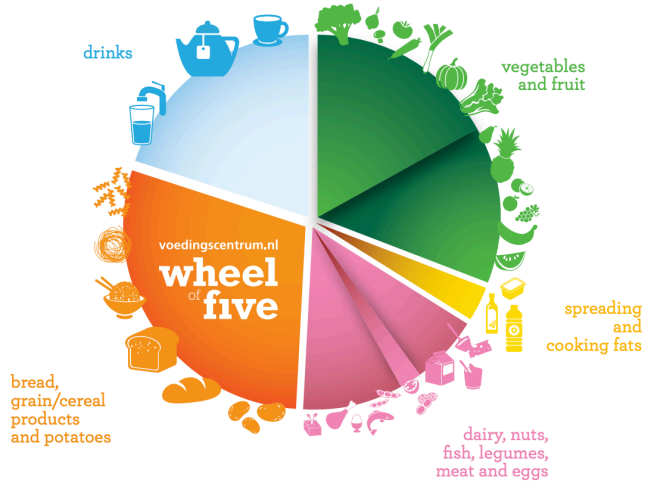

Figure 1. Dietary recommendations from the Netherlands Nutrition Center

FUTURE PERSPECTIVES

Although the results described in this thesis contribute to current knowledge on determinants of body fat distribution, they need to be confirmed in larger prospective studies, or in randomized controlled trials. Below we describe recommendations for future research in the field of nutritional epidemiology.

Mendelian randomization: the solution to confounding and reverse causation?

As described above, results from observational epidemiological studies might sometimes be confounded and not completely accurate, despite our best efforts to minimize confounding by improving the design and statistical analyses from the study. A potential solution for this problem is to perform a Mendelian randomization study. This type of study, based on Mendel's laws of inheritance, uses genetic variants that are associated with the exposure rather than the exposure itself [27]. Genes are randomly

assorted from parents to offspring, a process that occurs during the formation of gametes and conception. Inheritance of a certain genetic variant is therefore completely random and does not, for example, depend on lifestyle decisions made later on in life. This way, the association between the genetic variant and the disease resembles the association between an exposure and the disease, but it does not suffer from reverse causation or confounding to which a conventional observational study might be susceptible [27]. Whereas randomized trials can often only assess short term effects, a Mendelian randomization study is particularly useful for studying lifetime exposures, such as dietary intake. Several previous studies have therefore used this concept in order to study causal effects of lifestyle factors such as consumption of dairy, cruciferous vegetables and alcohol on varying health outcomes [28]. As such, alcohol consumption has been shown to be causally related to oesophageal cancer risk by the use of genetic data on the aldehyde dehydrogenase 2 family (ALDH2) gene in a Mendelian randomization study [29]. Moreover, consumption of dairy and cruciferous vegetables has been causally linked to cancer [30].

Following the research outlined in this thesis, Mendelian randomization studies could also be used to further investigate whether the associations we described are indeed causal. For example, a genome wide association study on inter-individual variation in dietary macronutrient intake in almost 300,000 participants has been conducted and has identified 96 genome-wide significant loci [31]. Such loci have also been reported in relation to dietary pattern scores, although the population was smaller [32]. These loci can be used to investigate to what extent macronutrient intake influences liver fat content. By using such genetic instruments, we would be able to study the effect of dietary habits on adiposity while limiting reverse causation and confounding by other lifestyle factors such as smoking, physical activity, stress, sleep habits or culture, provided a strong genetic scoring instrument is available and the number of participants is sufficient. Nevertheless, the analogy of Mendelian randomization with randomized controlled trials has strong implications for the design, reporting and interpretation, and therefore the use of this study design has several limitations to overcome in the future [33]. In the meantime, we have to rely on randomized controlled trials and observational studies to further explore the role of dietary habits and physical activity and visceral fat and liver fat.

Future of the NEO study

As described before, all research in this thesis has been performed in a cross-sectional setting and is based on the baseline measurements of all participants. The Netherlands Epidemiology of Obesity has been designed as a prospective cohort study and participants were included between 2008 and 2012. Since then, Dutch general practitioner databases have been used to collect information on incident diabetes, cardiovascular events or mortality. Moreover, it is planned that participants of the Netherlands Epidemiology of Obesity study will be invited to the study center for a second visit, during which multiple measurements and questionnaires will be taken and administered. Future research with these data will reveal whether changes in dietary intake or physical activity habits, the risk factors studied in this thesis, are also longitudinally associated with changes in visceral fat and liver fat, and the occurrence of cardiometabolic disease.

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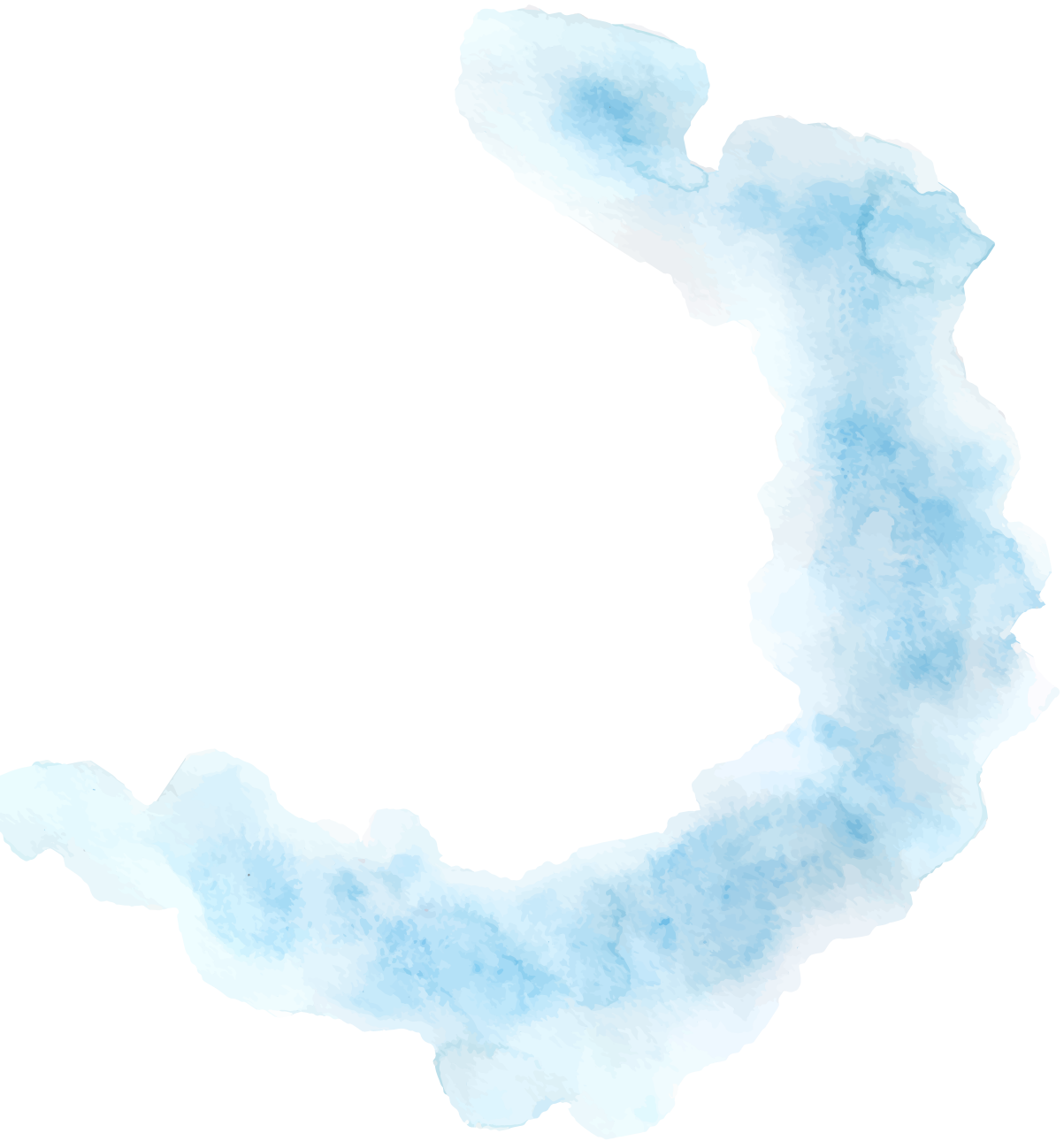

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APPENDICES

Nederlandse samenvatting

Dankwoord

Curriculum vitae

NEDERLANDSE SAMENVATTING

Overgewicht wordt gekenmerkt door een overmatige opslag van triglyceriden in vetcellen door een hogere inname dan verbruik van energie, wat uiteindelijk kan leiden tot gezondheidsproblemen. De Wereldgezondheidsorganisatie (WHO) definieert overgewicht op basis van de *body mass index* (BMI), die berekend wordt door het lichaamsgewicht te delen door de lengte in het kwadraat. Op basis van de BMI maakt de WHO onderscheid tussen verschillende categorieën van lichaamsgewicht: ondergewicht (BMI <18.5 kg/m^2), normaal gewicht (BMI 18.5-24.99 kg/m^2) en overgewicht (BMI > 25.0 kg/m^2). De laatstgenoemde categorie kan verder worden onderverdeeld in preobesitas (25.0-29.99 kg/m^2), obesitas klasse 1 (30.0-34.99 kg/m^2), obesitas klasse 2 (35.0-39.99 kg/m^2) en obesitas klasse 3 (>40 kg/m^2).

De afgelopen decennia komt obesitas steeds vaker voor. In 1980 had wereldwijd ongeveer 25% van alle mensen overgewicht, van wie meer dan 6% obesitas. Deze percentages zijn gestegen tot 34 en 12 in 2008. In absolute getallen betekent dit een toename van 572 miljoen volwassenen met overgewicht in 1980 naar 1.46 miljard in 2008, van wie 508 miljoen met obesitas. Dit cijfer wordt verwacht nog verder te stijgen in de toekomst, tot ongeveer 2.2 miljard volwassenen met overgewicht na 2020. Deze trend is ook zichtbaar in Nederland: in 2018 had ongeveer de helft van alle Nederlandse volwassenen overgewicht, en maar liefst 15% obesitas.

Als gevolg van deze stijging van mensen met overgewicht en obesitas, is er ook een stijging in het voorkomen van allerlei chronische welvaartsziekten waarneembaar. Obesitas gaat namelijk samen met een verhoogd risico op het ontwikkelen van diabetes (suikerziekte) en cardiovasculaire aandoeningen zoals hoge bloeddruk, hartinfarcten en beroertes . Geschat wordt dat een BMI van 25 of hoger verantwoordelijk is voor 34% van alle gevallen van hoge bloeddruk in mannen, en maar liefst 62% in vrouwen. Een BMI van 40 of hoger leidt zelfs tot een zeven keer zo hoog risico op het ontwikkelen van diabetes type 2 als een BMI tussen de 18,5 en 25. Hiernaast kan een hoge BMI ook leiden tot een verhoogd risico op verschillende soorten kanker. Ook gaat het samen met nierziekten, artrose, dementie en allerlei angst- en gedragsstoornissen.

Hoewel de BMI een makkelijke en veelgebruikte maat is voor de hoeveelheid lichaamsvet, die er enigszins mee rekening houdt dat lichaamslengte samenhangt met gewicht, houdt het geen rekening met de samenstelling van het lichaam. Zo wordt er bijvoorbeeld geen onderscheid gemaakt tussen vetmassa en vetvrije massa, zoals spiermassa, botmassa en water. Zo kunnen zeer getrainde mensen een relatief hoge BMI hebben, en toch weinig lichaamsvet. Hiernaast zegt de BMI ook niets over waar in het lichaam het vet precies is opgeslagen. Reeds halverwege de vorige eeuw werd duidelijk

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dat naast de hoeveelheid vet ook de plaats waar het lichaamsvet wordt opgeslagen van belang is: epidemiologen namen waar dat mensen met een zogenaamd ´appelfiguur´ bij wie het vet voornamelijk op buikhoogte is opgeslagen, een hoger risico hebben op het ontwikkelen van hart- en vaatziekten dan mensen met een zogenaamd ´peerfiguur´, bij wie het vet voornamelijk op de heupen of bovenbenen is opgeslagen. Door middel van een computed tomography (CT) scan konden Japanse onderzoekers niet veel later onderscheid maken tussen verschillende soorten vet op buikhoogte : onderhuids vet, ofwel subcutaan vet, bevindt zich vlak onder het huidoppervlak, terwijl visceraal vet dieper in de buikholte rondom de organen ligt opgeslagen. Ongeveer 82 tot 97% van het lichaamsvet is subcutaan vet, en 10 tot 15% bestaat uit visceraal vet.

Waar het lichaamsvet precies wordt opgeslagen, is afhankelijk van verscheidene factoren. Zo hebben vrouwen doorgaans bij eenzelfde BMI meer lichaamsvet dan mannen, maar minder visceraal vet. Geslachtshormonen lijken hierbij een belangrijke rol te spelen, aangezien bij vrouwen het visceraal vet vaak toeneemt na de overgang. Ook bepaalde genen, leeftijd en etniciteit gaan samen met de ophoping van visceraal vet.

Naast opslag in het vetweefsel kan overtollig vet ook ophopen op plaatsen in het lichaam waar het niet hoort, bijvoorbeeld in en rond bepaalde organen, zoals de lever of het hart. Dit wordt ook wel ectopisch vet genoemd. Volgens de zogenaamde '*lipid overflow hypothese*' wordt een overschot aan calorieën gewoonlijk in het onderhuidse vetweefsel opgeslagen, wat als gevolg hiervan kan uitzetten. Als dit vetweefsel echter niet naar behoren functioneert of onvoldoende capaciteit heeft om steeds meer vet op te slaan, worden de vetzuren opgeslagen in het viscerale vetdepot. Dit kan vervolgens leiden tot de afzetting van ectopisch vet, bijvoorbeeld in de lever. Een teveel aan vet in de lever zorgt voor een verstoord glucose- en vetzuurmetabolisme, wat kan leiden tot insulineresistentie (prediabetes) en samengaat met een verhoogd risico op type 2 diabetes en hart- en vaatziekten. Levervet en visceraal vet lijken de grote boosdoeners wat betreft het verhoogde risico op cardiometabole aandoeningen dat samengaat met obesitas.

Hierdoor kunnen visceraal vet en levervet ook een belangrijke rol spelen in het voorkomen van cardiometabole aandoeningen, namelijk wanneer we het zouden kunnen reduceren. Omdat er momenteel nog geen medicijnen bestaan om visceraal vet en levervet te verminderen, moet de nadruk hierbij liggen op leefstijlfactoren zoals gezonde voeding en lichamelijke beweging. Hoewel men hierbij vaak inzet op gewichtsverlies door het beperken van energie-inname, kan de kwaliteit van de voeding ook een belangrijke rol spelen. Het effect van voeding op de gezondheid kan op verschillende manieren onderzocht worden. Om te onderzoeken welke componenten van voeding precies verbonden zijn aan ziekte, is het van belang om naar de micro- en macronutriënten te kijken. Zo is de inname van fructose bijvoorbeeld geassocieerd aan vetophoping in de lever. Eerder is ook al aangetoond dat inname van verzadigd vet samengaat met meer levervet dan inname van onverzadigd vet. Voor visceraal vet bestond reeds een overzicht van de effecten van de macronutriëntsamenstelling van de voeding op de hoeveelheid visceraal vet door middel van een meta-analyse. Voor levervet bestond een dergelijk overzicht nog niet. In **Hoofdstuk 2** beschrijven we een systematische overzicht en meta-analyse van gerandomiseerde gecontroleerde studies naar de effecten van de macronutriëntsamenstelling van de voeding op de hoeveelheid levervet. Hierin konden we aantonen dat het vervangen van koolhydraten door eiwitten leidt tot een afname van de hoeveelheid levervet. Wanneer koolhydraten echter vervangen worden door vetten lijkt dit geen effect te hebben op de mate van leververvetting, hoewel de resultaten van verschillende studies in conflict zijn met elkaar. Dit zou kunnen komen doordat bij veel studies niet vermeld werd welke vetsoorten werden uitgewisseld, aangezien verzadigd vet leidt tot meer levervet dan (enkelvoudig of meervoudig) onverzadigd vet. Ook de bronnen van de voedingsvetten werden vaak niet vermeld (vlees, plantaardig of zuivel). Binnen de voedingsvetten lijkt het type vet wel uit te maken: het vervangen van onverzadigd vet door verzadigd vet leidt tot een grotere mate van leververvetting. Aangezien slechts een beperkt aantal studies geschikt was om mee te nemen in deze meta-analyse, hebben we een belangrijk gat in de huidige kennis geïdentificeerd. Op basis hiervan adviseren we dat vooral meer studies uitgevoerd moeten worden die de uitwisseling van vetten en eiwitten met elkaar vergelijken, maar zeker ook onderzoeken die een vergelijking maken tussen zowel vetten, eiwitten als koolhydraten. Hierbij is het van groot belang dat rekening gehouden wordt met de bron van de macronutriënten (dierlijk of plantaardig) en het type ervan (meervoudig onverzadigd vet versus enkelvoudig onverzadigd vet).

Vervolgens hebben we voeding op verschillende niveaus bestudeerd in de Nederlandse Epidemiologie van Obesitas (NEO) studie. Dit is een groot cohort van meer dan 6500 mannen en vrouwen van middelbare leeftijd uit Leiden en omgeving. Aangezien voeding meer is dan enkel de som van voedingsstoffen kan het voor de klinische praktijk nuttig zijn om voedingspatronen als een geheel te bestuderen. Interactie tussen voedingsstoffen kan namelijk een rol spelen in de effecten van deze voedingsstoffen op de gezondheid. Deze recente inzichten hebben geleid tot voedingsrichtlijnen in zowel Europese landen als de Verenigde Staten, die zijn gebaseerd op voedingsproducten en -groepen in plaats van voedingsstoffen. Onderzoek heeft laten zien dat voedingsgroepen zoals vlees, zuivel en groente en fruit samengaan met lichaamsgewicht, diabetes

en cardiometabole aandoeningen. Het is echter nog niet bekend in hoeverre deze voedingsgroepen ook specifiek samengaan met de hoeveelheid visceraal vet en levervet. Dit hebben wij in **Hoofdstuk 3** onderzocht, en we beschrijven hierin onze bevindingen omtrent de voedingsinname van zuivel, vlees, vis, fruit en groente, plantaardige vetten en oliën, en zoete snacks. Aangezien visceraal vet en levervet sterk samengaan met totaal lichaamsvet, en bij een hogere inname uit een bepaalde voedingsgroep de toename van visceraal vet of levervet ook louter een resultaat kan zijn van toegenomen totaal lichaamsvet, is het belangrijk om hier rekening mee te houden. In onze analyses hebben we dan ook gecorrigeerd voor totaal lichaamsvet, zodat we konden onderzoeken in hoeverre de inname van bepaalde voedingsgroepen specifiek samengaan met visceraal vet en levervet. Zelfs na deze correctie bleek dat een toename in consumptie van 100 gram per dag van groente en fruit samengaat met een verminderde hoeveelheid visceraal vet van 1.2 cm^2. Hiernaast gaat een consumptie van 100 gram per dag van zoete snacks, bovenop de gebruikelijke voeding, samen met een relatieve toename in levervet van ongeveer 20 procent.

Ook Nederland heeft naar aanleiding van de recente verschuiving van voedingsstoffen naar voedingsgroepen zijn voedingsrichtlijnen aangepast. In 2015 heeft de Gezondheidsraad nieuwe Richtlijnen Goede Voeding uitgebracht. Hierin wordt een meer plantaardige en minder dierlijke voeding geadviseerd, en er worden verschillende adviezen gegeven op basis van 15 componenten. Zo wordt onder andere geadviseerd om: minstens 200 gram groente en 200 gram fruit te eten, enkele porties zuivel, en de consumptie van rood en bewerkt vlees te beperken. Om in kaart te brengen hoe goed mensen zich aan deze richtlijnen houden, is de Dutch Healthy Diet (DHD) index ontwikkeld. De score op deze index kan uiteenlopen van 0 tot 150, waarbij een hogere score een betere naleving van de richtlijnen betekent. In **Hoofdstuk 4** hebben we onderzocht of een betere naleving van de richtlijnen samengaat met minder lichaamsvet. We vonden dat 10 punten hoger op de index inderdaad samenging met een absolute vermindering in totaal lichaamsvet van 0.2 procent. Ook ging het samen met 2.3 cm^2 minder visceraal vet en relatief gezien 6 procent minder levervet, ook als we rekening hielden met de hoeveelheid totaal lichaamsvet. Dit verband leek niet zozeer het gevolg van één van de 15 componenten in het bijzonder, aangezien het één voor één weglaten van de componenten niet leidde tot grote veranderingen in het verband. Op basis hiervan kunnen we dan ook concluderen dat het belangrijk is om een algeheel gezond voedingspatroon aan te hangen zoals in de richtlijnen van de Gezondheidsraad wordt beschreven.

Overmatig alcoholgebruik is een welbekende risicofactor voor leververvetting. De huidige richtlijnen voor het voorkomen of verminderen van leververvetting stellen dat overmatig alcoholgebruik vermeden dient te worden. Echter het is nog niet bekend met welke non-alcoholische dranken deze alcoholische dranken het beste vervangen kunnen worden indien iemand wordt aangeraden om te stoppen met drinken. Energiehoudende dranken zoals frisdrank kunnen natuurlijk ook door een teveel aan calorieën leiden tot leververvetting. In **Hoofdstuk 5** hebben we onderzocht hoe verschillende alcoholische en non-alcoholische dranken samengaan met levervet. We zagen dat ieder extra glas alcohol samengaat met meer levervet, en melk, koffie en thee met minder levervet. In een isocalorisch substitutiemodel hebben 5 energieprocent alcoholische dranken uitgewisseld tegen 5 energieprocent non-alcoholische dranken, en zo rekening gehouden met het aantal calorieën dat een bepaalde drank bevat. Uit deze analyses bleek dat het vervangen van alcohol door melk samengaat een relatieve afname van levervet van 12 procent, maar het vervangen van alcohol door suikerhoudende dranken ging niet samen met minder levervet. Op basis van deze resultaten lijken het vooral de suikers te zijn die samenhangen met levervet, meer dan de energie op zich. Op basis hiervan concluderen we dat het niet aan te raden is om alcohol met suikerhoudende dranken als sap of frisdrank te vervangen bij een advies om te stoppen met het drinken van alcohol, maar wel door bijvoorbeeld melk, koffie thee of water.

Naast voeding is ook lichaamsbeweging een belangrijke te beïnvloeden risicofactor van de hoeveelheid visceraal vet en levervet. In verscheidene studies is aangetoond dat een gebrek aan lichaamsbeweging kan leiden tot een toename in lichaamsgewicht, en daarbij ook in visceraal vet en levervet. Teveel tijd zittend doorbrengen gaat ook samen met meer lichaamsvet, zelfs wanneer rekening wordt gehouden met de hoeveelheid lichaamsbeweging. In **Hoofdstuk 6** beschrijven we hoe een subgroep van de NEO deelnemers gedurende enkele dagen een Actiheart monitor heeft gedragen om hun lichaamsbeweging en zitgedrag in kaart te brengen. Deze monitor meet de hartslag en de beweging van de deelnemers, die wij gecombineerd hebben tot een activiteitsscore. De intensiteit van lichaamsbeweging wordt weergegeven in metabool equivalenten van een taak (MET). Alle activiteiten onder de 1.5 MET worden als zittend gedrag beschouwd, activiteiten met een MET score tussen de 1.5 en 3 als lichte activiteiten, tussen de 3 en de 6 als gematigde activiteiten en alles boven de 6 MET als intensief. Aangezien er 24 uren in een dag zitten, betekent minder tijd zittend doorgebracht vanzelf meer tijd in beweging. Of het voor het viscerale vet en levervet uitmaakt of je zitten nu met lichte, matige of intensieve lichaamsbeweging vervangt, is nog niet goed onderzocht. Om deze vraagstelling te beantwoorden, hebben we isotemporele substitutieanalyses uitgevoerd, waarin we het vervangen van een half uur per dag zitten door een half uur van lichte of gematigde tot intensieve lichaamsbeweging modelleren. Hieruit bleek dat het vervangen van een half uur zitten door lichte activiteit niet samenging

met minder lichaamsvet, visceraal vet of levervet, en het vervangen door een half uur gematigde tot intensieve activiteit wel. Wanneer we de verbanden echter corrigeerden voor totaal lichaamsvet om te onderzoeken of de verbanden specifiek waren, verdwenen deze voor visceraal vet en levervet. Op basis hiervan concluderen we dat er geen extra verband is tussen lichaamsbewegingen visceraal vet en levervet, bovenop het effect van lichaamsbeweging op totaal lichaamsvet.

Tot slot bediscussiëren we de bevindingen uit dit proefschrift in **Hoofdstuk 7**, en de voor- en nadelen van de gebruikte onderzoeksopzetten. Hierbij gaan we ook in op de klinische implicaties en de mogelijke vooruitzichten voor toekomstig onderzoek. Concluderend kunnen we stellen dat het onderzoek beschreven in dit proefschrift heeft bijgedragen aan ons begrip over de relatie tussen leefstijl en visceraal vet en levervet, en op welke factoren we ons dienen te richten als het gaat om de preventie van ectopische vetophoping en cardiometabole aandoeningen.

DANKWOORD

Graag wil ik hierbij even stilstaan bij iedereen die op welke wijze dan ook betrokken is geweest bij de totstandkoming van dit proefschrift.

Allereerst gaat mijn dank uit naar mijn promotor **Prof. dr. Rosendaal** en mijn copromotor **dr. ir. De Mutsert**. Dankzij jullie heb ik de kans gekregen mij als volwaardig wetenschapper te ontwikkelen. Jullie inzichten samen met de adviezen van alle co-auteurs hebben geleid tot een mooi en samenhangend proefschrift.

Aan het NEO management team, bedankt voor al jullie harde werk om deze mooie studie draaiende te houden. Alle collega's van de afdeling Klinische Epidemiologie wil ik bedanken voor de samenwerking en informele werksfeer. Tijdens de wekelijkse meetings op dinsdag heb ik van jullie allemaal mogen leren en heb ik mijn eigen blik op onderzoek kunnen ontwikkelen. To all my fellow PhD colleagues from the department of Clinical Epidemiology, thank you so much for everything. The past 4 years you always made me feel at home, and I'm very grateful for that.

Al mijn vrienden en familie, bedankt voor jullie ongeveinsde interesse en het aanhoren van al mijn (waarschijnlijk onbegrijpelijke) verhalen over mijn werk. Bijzonder fijn om zo'n netwerk te hebben waar je altijd op terug kunt vallen.

Sebastiaan, mijn hele promotietraject heb ik samen met jou doorlopen. Van Energise tot TKI, van cursussen tot internationale congressen en van Cambridge tot Wenen. Geweldig dat je ook tijdens mijn verdediging naast me wil staan, ik had me geen betere paranimf kunnen wensen. **Cynthia**, enorm bedankt voor al je hulp en afleiding wanneer we dit allebei even nodig hadden. Wat een geweldige afsluiting van onze promotietijd om elkaars paranimf te zijn.

Hans en Angely, hoewel je je schoonouders niet kunt kiezen, had ik het niet beter kunnen treffen. Bedankt dat jullie mij met open armen hebben opgenomen, ik heb me altijd welkom gevoeld. **Marjolein**, wat begon als een ontmoeting in Maastricht met borrelhapjes, is uitgegroeid tot een geweldige band. Je bent als de zus die ik nooit gehad heb, en ik ben dankbaar dat je in mijn leven bent gekomen.

Lieve **papa en mama**, jullie hebben altijd in mij geloofd, en steunden me bij iedere keuze die ik heb gemaakt. Wanneer het tegenzat kon ik altijd weer thuiskomen. Ik kan niet beschrijven hoeveel dit voor mij betekent. Dit proefschrift is voor jullie.

Liefste **Jan**, jouw aanwezigheid maakt alles mooier. Zonder jouw steun, geduld en relativerende woorden had dit proefschrift er nu niet gelegen. Ik ben er trots op jou mijn man te mogen noemen, en kijk uit naar alles wat ons verdere leven ons te bieden heeft.

CURRICULUM VITAE

Esther Winters-van Eekelen werd geboren op 24 december 1992 te Breda. In 2011 behaalde zij haar gymnasiumdiploma aan de Katholieke Scholengemeenschap Etten-Leur, waarna zij naar Maastricht verhuisde om daar aan haar bacheloropleiding Gezondheidswetenschappen te beginnen aan de Universiteit van Maastricht. Gedurende deze opleiding volgde zij de major Preventie & Gezondheid, en heeft ze een minor in Psychologie afgerond. Vervolgens heeft zij aan dezelfde universiteit de masteropleiding Epidemiologie gevolgd en zich gedurende de masterthesis verdiept in de Genetische en Moleculaire Epidemiologie. In 2016 begon zij als promovenda op de afdeling Klinische Epidemiologie in het Leids Universitair Medisch Centra onder begeleiding van dr. ir. Renée de Mutsert en Prof. dr. Frits Rosendaal. Gedurende dit promotietraject werkte zij mee aan onderzoek binnen de Nederlandse Epidemiologie van Obesitas (NEO) studie en was ze actief betrokken bij het opschonen van de data hiervan. Voor de registratie als Epidemioloog B volgde zij verschillende cursussen en bezocht (inter)nationale congressen om haar werk te presenteren. Ook heeft ze verscheidene studenten begeleid, en was ze voorzitter van de 'NEO journalclub' en een lid van het Young Talent Forum van het Energise Consortium. Momenteel werkt ze als postdoctoraal onderzoeker op de afdeling Klinische Epidemiologie in Leiden en helpt bij de totstandkoming van de tweede meting van NEO.

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PORTFOLIO

Oral and poster presentations	Year	Credits
Sweet Snacks Are Positively and Fruits and Vegetables Are Negatively Associated with Visceral or Liver Fat Content in Middle-Aged Men and Women. Papendal course from Dutch Heart Foundation, Papendal, the Netherlands.	2016	0,25
Sweet Snacks Are Positively and Fruits and Vegetables Are Negatively Associated with Visceral or Liver Fat Content in Middle-Aged Men and Women. ICCR Conference on Chronic Societal Cardiometabolic Diseases, Québec, Canada.	2017	0,50
Consumption of alcoholic and sugar sweetened beverages is associated with increased liver fat content in middle-aged men and women. ICCR Conference on Chronic Societal Cardiometabolic Diseases, Québec, Canada.	2017	0,50
Consumption of alcoholic and sugar sweetened beverages is associated with increased liver fat content in middle-aged men and women. WEON congress, Antwerp, Belgium.	2017	0,50
Sweet Snacks Are Positively and Fruits and Vegetables Are Negatively Associated with Visceral or Liver Fat Content in Middle-Aged Men and Women. Invited lecture on Translational Cardiovascular Research Meeting, Utrecht, the Netherlands.	2017	0,25
Consumption of alcoholic and sugar sweetened beverages is associated with increased liver fat content in middle-aged men and women. Annual Dutch Diabetes Research Meeting (NVDO-ADDRM), Oosterbeek, the Netherlands.	2017	0,25
Consumption of alcoholic and sugar sweetened beverages is associated with increased liver fat content in middle-aged men and women. Papendal cursus van Hartstichting, Papendal, the Netherlands.	2017	0,25
Adherence to dietary guidelines in relation to visceral fat and liver fat in middle-aged men and women: the NEO study. WEON congres, Bilthoven, the Netherlands.	2018	0,50
Adherence to dietary guidelines in relation to visceral fat and liver fat in middle-aged men and women: the NEO study. Translational Cardiovascular Research Meeting, Utrecht, the Netherlands.	2018	0,25
Adherence to dietary guidelines in relation to visceral fat and liver fat in middle-aged men and women: the NEO study. Dutch Nutritional Science Days, Heeze, the Netherlands.	2018	0,25
Adherence to dietary guidelines in relation to visceral fat and liver fat in middle-aged men and women: the NEO study. European Congress on Obesity (ECO), Vienna, Austria.	2018	0,50

Adherence to dietary guidelines in relation to visceral fat and liver fat in middle-aged men and women: the NEO study. Annual Dutch Diabetes Research Meeting (NVDO-ADDRM), Oosterbeek, the Netherlands.	2018	0,25
Effects of dietary macronutrients on liver fat content in adults: a systematic review and meta-analysis of randomized controlled trials, WEON congres, Groningen, the Netherlands.	2019	0,50
Reallocation of sedentary time to moderate to vigorous physical activity associated with reduced total body fat, visceral fat and liver fat. Annual Dutch Diabetes Research Meeting (NVDO-ADDRM), Wageningen, the Netherlands.	2019	0,25

Consortia

Top Kennis Instituut (TKI) liver fat in collaboration with Unilever and Maastricht University	2017-2019	3,00
Energise! Consortium from the Dutch Heart Foundation	2016-2019	3,00

Congresses and symposia

Dairy Matrix Symposium	2017	0,25
ICCR Conference on Chronic Societal Cardiometabolic Diseases, Québec, Canada.	2017	0,75
Netherlands Association for the Study of Obesity (NASO) Spring meeting, Utrecht, the Netherlands.	2017-2019	0,75
WEON congres (Antwerpen, Bilthoven, Groningen, the Netherlands)	2017-2019	1,50
Annual Dutch Diabetes Research Meeting, Oosterbeek, the Netherlands.	2017-2019	1,00
Translational Cardiovascular Research Meeting, Utrecht, the Netherlands.	2017-2018	1,00
European Congress on Obesity (ECO), Vienna, Austria.	2018	1,00
Dutch Nutritional Science Days, Heeze, the Netherlands.	2018	0,50

Courses, seminars and master classes

Introduction to Clinical Epidemiology (Rothman), Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands	2016	2,00
Basic Course Legislation and Organization for Clinical Researchers (BROK/GCP), Graduate School, Leiden University Medical Center, the Netherlands	2016	1,00
Basic methods and reasoning in biostatistics, Boerhaave Instituut, Leiden, the Netherlands	2016	1,50
Nutritional Epidemiology, Epidemiology Graduate School of Life Sciences, Utrecht University, the Netherlands	2016	1,50
Clinical Epidemiology Schiermonnikoog, Boerhaave Instituut, Leiden, the Netherlands	2016	2,00
Clinical Epidemiology (Grobbee), Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2016	3,00
Atherosclerosis and Thrombosis, Papendal Hartstichting Course, Papendal, the Netherlands	2016	1,50
Statistical Aspects of Clinical Trials, Boerhaave Instituut, Leiden, the Netherlands	2017	1,00
Survival Analyses, Boerhaave Instituut, Leiden, the Netherlands	2017	1,50
Cardiac Function and Adaptation, Papendal Hartstichting Course, Papendal, the Netherlands	2017	1,50
Causal Inference (Hernan), Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands	2017	3,00
Systematic Reviews and Meta-analyses, Boerhaave Instituut, Leiden, the Netherlands	2018	1,00
Physical activity measurement seminar, Cambridge, United Kingdom	2018	1,50
Regression Analyses, Boerhaave Instituut, Leiden, the Netherlands	2018	1,50
Causal Inference (Hernan) Journal Club, Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands	2018	1,00
Weekly Research Lunch,Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2016-2019	3,00
Weekly Capita Selecta, Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2016-2019	3,00
Bi-weekly Journal Club from NEO study, Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2016-2019	3,00

Student monitoring and teaching

Master thesis Babette de Roos	2018	1,50
Supervision visiting researcher Laura Martin-Piedra	2019	1,00
Master thesis Eleonora Bassetti	2019-2020	1,00
Teacher for Introduction to Clinical Epidemiology (Rothman) for students, Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2017	2,00
Various epidemiological classes for bachelor and master students biomedical sciences and medicine, Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2016-2020	5,00

Other activities

Reviewing scientific epidemiological publications	2017-2019
Chair of the NEO journal club, Leiden, the Netherlands	2017-2019
Co-organizing the 2-day bi-annual Energise! Consortium meeting, Leiden, the Netherlands	2018
Member of the Energise! Consortium Young Talent Forum	2018-2019