Effects of dietary fat on cholesterol efflux and other cardiometabolic risk markers in humans

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– focus on palmitic acid and stearic acid –

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Content

CHAPTER 1

General introduction

Cholesterol is essential for the human body as it is a constituent of most membranes, is needed for bile acid synthesis, and is a precursor for steroid hormones and vitamin D. However, too much cholesterol is harmful, because it can accumulate in macrophages within the arterial wall and thereby contribute to the development of atherosclerosis. Atherosclerosis, the build-up of fatty substances in the arterial wall, is the most common cause of cardiovascular diseases (CVD), an umbrella term for diseases related to heart and blood vessels such as coronary heart disease (CHD). CHD is the consequence of disrupted blood flow to the heart due to fat deposits in the coronary arteries. The development of the so-called atherosclerotic plaque is a complex process that involves endothelial dysfunction, inflammation, proliferation of smooth muscle cells and connective tissue, and the accumulation of lipids such as cholesterol [1]. Under normal conditions, macrophages export their excess cholesterol, but when the balance between cholesterol influx and efflux is disturbed, large deposits of esterified cholesterol are formed and these macrophages transform into lipid-loaded foam cells [2]. Our diet, in particular dietary fat intake, can influence the risk of developing atherosclerosis.

Dietary fat

The macronutrients fat, protein and carbohydrates provided by our diet are necessary to fuel our body and to keep all organs vital. Of these, fat has the highest energy density, i.e. 9 kilocalories per gram compared with 4 kilocalories per gram for proteins or carbohydrates. Besides providing energy, fats have an important role as building block of cell membranes and as precursor for hormones and other bioactive molecules. In addition, the vitamins A, D, E, and K can only be absorbed when delivered with fat. The diet provides different types of fat, such as cholesterol and phospholipids, but the majority of dietary fats are the triacylglycerols, also called triglycerides, which account for more than 95% of total dietary fat. These triacylglycerols consist of a glycerol backbone to which three fatty acids are attached as depicted in **Figure 1**. Triacylglycerols contain a mixture of different fatty acids, which differ in the amount of saturation (saturated, monounsaturated, or polyunsaturated) and chain length. It is generally believed that unsaturated fatty acids are healthier than saturated fatty acids (SFA), with the exception of monounsaturated fatty acids (MUFA) in the *trans* configuration (*trans* fat). This is mainly based on the negative effects of saturated and *trans* fatty acids on serum low-density lipoprotein (LDL)-cholesterol concentrations [3], since elevated serum LDL-cholesterol concentrations are a well-established risk factor for atherosclerosis [4]. LDL-cholesterol is therefore also known as the 'bad' cholesterol.

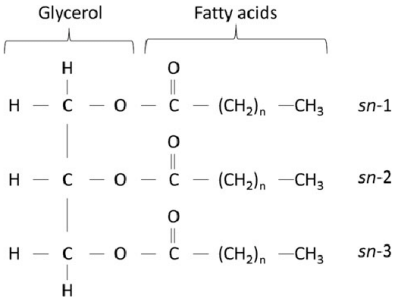

Figure 1. Schematic drawing of a triacylglycerol molecule.

Sources and intakes of dietary fat

Dietary fat is present in almost all foods, both from animal and plant origin. Foods always contain a mixture of fatty acids – both saturated and unsaturated – but animal products such as meat and dairy products are generally high in SFA, while plant products such as vegetable oils are generally high in unsaturated fatty acids. Dietary guidelines recommend to consume 15 to 35 % of total daily energy (en%) from fat [5]. However, based on the effects on serum LDL-cholesterol, it is advised to keep the intake of SFA below 10 en%. In the Netherlands, the average intake of saturated fat is 12.6 en% [6]. The most commonly consumed SFA are palmitic acid (C16:0; hexadecanoic acid) and stearic acid (C18:0; octadecanoic acid), accounting for approximately 50 and 25% of total SFA respectively according to data from the Rotterdam study [7]. Thus, approximately 6% of total daily energy comes from palmitic-acid intake and 3% from stearic-acid intake. Palmitic acid is predominantly found in meat and dairy products such as butter, cheese, and milk but also some vegetable oils such as palm oil contain a high amount of palmitic acid. Cocoa butter is for example rich in stearic acid, but the major daily sources of stearic-acid intake are also meat and dairy products.

Absorption and transport of dietary fat

Upon ingestion of fat, the lipid droplets first become smaller by chewing and peristaltic movements of the stomach. When the lipid droplets enter the small intestine, bile – produced by the liver from cholesterol – emulsifies the fat, meaning that it further reduces the droplets to very small particles and also helps to make them water soluble (hydrophilic) as fat is insoluble in water (hydrophobic). Then, lipases – specific lipid-directed enzymes produced by the pancreas – hydrolyze the triacylglycerols into free fatty acids and a (monoacyl)glycerol molecule. The free fatty acids and (monoacyl)glycerols spontaneously form a micelle together with phospholipids, bile acids, and cholesterol. These micelles are taken up by the intestinal cells (enterocytes). Because fat is hydrophobic, it needs to be transported through the blood via so called lipoproteins (**Figure 2**). Our body can produce different types of lipoproteins. Upon intake of dietary fat, enterocytes produce chylomicrons,

which are a specific type of lipoprotein that can be recognized due to their apolipoprotein (apo) B48. Enterocytes secrete the chylomicrons into the lymph and these chylomicrons eventually end-up in the blood stream to transport the dietary fats through the rest of the body [8].

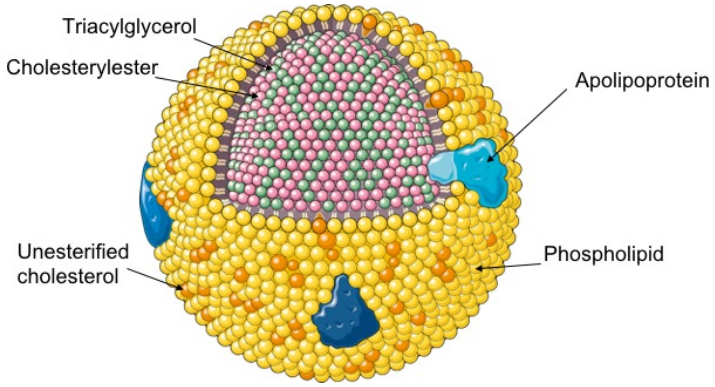

Figure 2. Schematic overview of a lipoprotein. Obtained from Servier Medical Art (https://smart.servier.com).

Lipoprotein metabolism

Besides chylomicrons, also other lipoproteins are produced by the body that transport triacylglycerols and cholesterol molecules through the circulation. Lipoproteins are heterogenic particles that differ in size and composition. In general, a lipoprotein consists of a hydrophobic core containing cholesteryl esters and triacylglycerols, and an (partly) hydrophilic outer surface consisting of phospholipids, apolipoproteins, and free cholesterol (**Figure 2**). Lipoproteins are generally classified as chylomicrons, very-low density lipoproteins (VLDL), low-density lipoproteins (LDL), or high-density lipoproteins (HDL). The different lipoproteins have different functions. Chylomicrons and VLDL particles predominantly deliver triacylglycerols to the tissues, while LDL and HDL particles are more important for cholesterol transport. As mentioned before, chylomicrons are produced in the intestine by enterocytes and transport dietary lipids. In the circulation, the enzyme lipoprotein lipase (LPL) hydrolyses the triacylglycerols present in the chylomicrons which results in the delivery of fatty acids to different tissues such as the heart and muscles. Upon hydrolysis by LPL, the size of the chylomicron reduces and eventually a so-called remnant, which has now become relatively cholesterol-rich, is left. Chylomicron remnants can be taken-up by the liver. In the liver, VLDL particles are produced by hepatocytes. Like chylomicrons, VLDL particles are large particles rich in triacylglycerols (triacylglycerol-rich lipoproteins; TRLs) that deliver fatty acids to tissues via the action of LPL. In the circulation, VLDL particles become smaller due to hydrolysis and can either be taken up by the liver via the LDL-receptor or remain in the circulation as LDL,

which in turn can be endocytosed by various cells to donate cholesterol. Hepatocytes and enterocytes also produce apolipoprotein A-I (apoA-I) molecules that can bind to phospholipids and form pre-β-HDL particles. In the circulation, these pre-β-HDL particles become enriched with cholesterol, which is converted into cholesteryl esters by lecithin-cholesterol acyltransferase (LCAT) for efficient storage within the lipoprotein, and eventually form a mature HDL particle. HDL particles can take-up cholesterol from the periphery and return it to the liver. This will result in either the removal of cholesterol from the body via excretion in bile or conversion into bile acids, or the cholesterol can re-enter the circulation via production of VLDL. This process is called reverse cholesterol transport. HDL particles can deliver cholesteryl esters directly to the liver via interaction with the scavenger receptor class B member 1 (SR-B1) or indirectly via cholesterol transfer to VLDL and LDL. Indirect reverse cholesterol transport is mediated by cholesteryl ester transport protein (CETP). CETP exchanges cholesteryl esters from HDL particles to VLDL and LDL particles, and triacylglycerols from VLDL and LDL particles to HDL particles. In this way, the VLDL and LDL particles get enriched with cholesterol. In addition, the triacylglycerols now present in HDL can be hydrolyzed by hepatic lipase and the remaining cholesterol-depleted HDL particles (HDL_3 particles) can accept cholesterol from the periphery again [8].

HDL-mediated cholesterol efflux capacity

Many epidemiological studies have shown an inverse relationship between concentrations of HDL-cholesterol and CVD [9]. Therefore, HDL-cholesterol is considered as 'good cholesterol'. However, this inverse relationship has recently been debated, because pharmacological studies that increased HDL-cholesterol concentrations with CETP-targeted drugs have failed to reduce cardiovascular events [10-12]. Thus, the relationship between HDL-cholesterol and CVD does not seem to be causal and the focus has shifted more towards the functionality of HDL particles. HDL particles have multiple anti-atherogenic functions, among which the ability to accept cholesterol from the periphery via cholesterol efflux (cholesterol efflux capacity; CEC). HDL-mediated cholesterol efflux is the first step of – and thus crucial for – the reverse cholesterol transport pathway. In general, cholesterol efflux is the movement of cholesterol from a peripheral cell (cholesterol donor) to a cholesterol acceptor. Related to atherosclerosis, HDL-mediated cholesterol efflux from lipid-loaded macrophages – for example present in the arterial wall – is particularly important. Macrophages can transfer cholesterol via four different pathways, including a) passive aqueous diffusion; b) passive diffusion mediated by SR-B1; c) active transport mediated by ATP-binding cassette transporter (ABC) A1; or d) active transport mediated by ABCG1 [13]. In lipid-loaded macrophages, the predominant pathway is ABCA1-mediated cholesterol efflux [14]. ApoA-I interacts with the ABCA1-receptor and this interaction results in the efflux of cholesterol and some phospholipids from macrophages to lipid-poor apoA-I (pre-β-HDL) [15]. ABCA1-mediated cholesterol efflux is inversely associated with atherosclerosis [16] and cardiovascular

events [17, 18]. This inverse relationship has also been demonstrated independently of concentrations of HDL-cholesterol and apoA-I [18]. Therefore, it appears that improving HDL functionality and specifically HDL-mediated cholesterol efflux via ABCA1 is a better target for decreasing CVD risk than simply increasing HDL-cholesterol.

A schematic overview of the lipoprotein metabolism including cholesterol efflux is shown in the figure below (**Figure 3**).

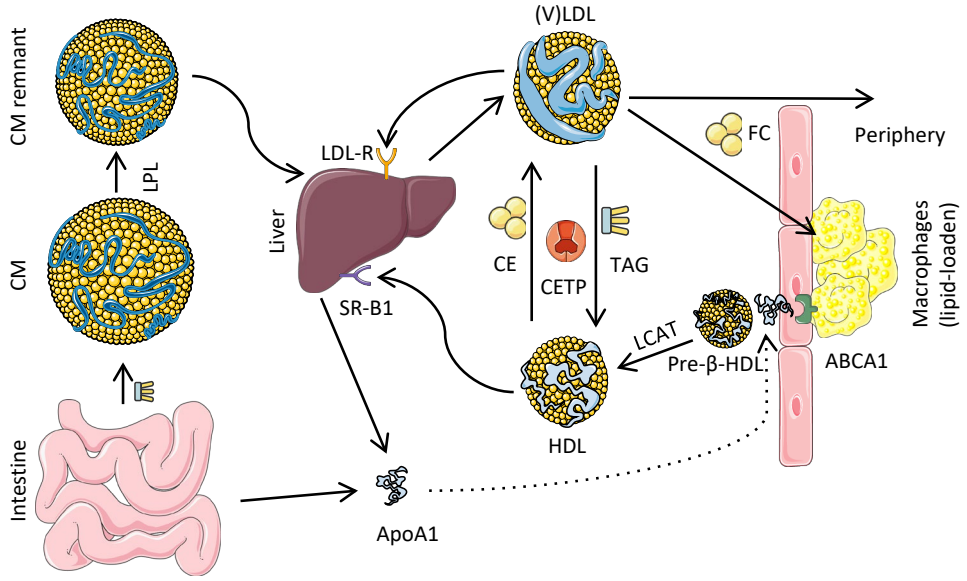

Figure 3. Schematic overview of lipoprotein metabolism and the reverse cholesterol transport pathway including cholesterol efflux from macrophages to pre-ß-HDL. Figure was created using Servier Medical Art (https://smart.servier.com). Abbreviations: apo, apolipoprotein; ABCA1, ATP-binding cassette transporter A1; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CM, chylomicron; FC, free cholesterol; HDL, high-density lipoprotein; LCAT, lecithin-cholesterol acyltransferase; LDL-R, low-density lipoprotein receptor; LPL, lipoprotein lipase; SR-B1, scavenger receptor class B member 1; TAG, triacylglycerol; VLDL, very-low density lipoprotein.

Effects of dietary fat on lipoprotein metabolism and cholesterol efflux

Dietary fat is known to affect serum cholesterol levels. Saturated and *trans* fatty acids are considered cholesterol-raising as compared with an iso-energetic amount of carbohydrates, while *cis*-unsaturated fatty acids are cholesterol-lowering [3]. However, not all SFA are equally cholesterol-raising. An exception is stearic acid (C18:0), which seems to have a neutral effect on serum cholesterol levels. Multiple studies have shown that palmitic acid (C16:0) raises serum concentrations of LDL-cholesterol and HDL-cholesterol compared with stearic acid [19]. The reason for this difference is still not completely understood. It has been suggested that the absorption of stearic acid is less than palmitic acid. Indeed, Baer and colleagues showed that 94% of stearic acid was absorbed versus 97% of palmitic acid. However, this difference is too small to explain the different effects on lipoprotein metabolism [20]. Another explanation may be the higher conversion of stearic acid into oleic acid (C18:1), thereby mimicking the effects of MUFA. However, only a small part – approximately 10% [21, 22] – of stearic acid is converted, which also makes it unlikely that this explains the metabolic differences between palmitic acid and stearic acid. Besides these minor differences in absorption and conversion, it is also possible that palmitic acid and stearic acid differently affect cellular cholesterol levels. Cellular cholesterol levels are tightly regulated via endogenous cholesterol synthesis and cholesterol absorption via the LDL-receptor. It has been shown that MUFA and polyunsaturated fatty acids (PUFA) increase the expression of the LDL-receptor compared with SFA, thereby enhancing clearance of both VLDL and LDL particles and decreasing cholesterol levels in the circulation [23]. The chain length of SFA has also been positively associated with the expression of the LDL-receptor, meaning that stearic acid upregulates the LDL-receptor compared with palmitic acid. However, this has only been shown in *in vitro* studies and in animal studies, but not in humans [23]. Why HDL-cholesterol concentrations are lower on a stearic acid-rich diet compared with a palmitic acid-rich diet is not entirely clear yet, but there are indications that CETP plays a role [24-26]. Another possible route is via decreased cholesterol efflux from macrophages to HDL particles resulting in lower HDL-cholesterol. However, if and how dietary fatty acids affect HDL-mediated cholesterol efflux is not yet fully understood and results are contradictive [27-30]. Since palmitic and stearic acids are predominant in the Western diet and have different effects on HDL-cholesterol, it is interesting to study their effects on HDL-mediated cholesterol efflux via ABCA1.

Postprandial metabolism and effects of dietary fat

As most people consume multiple meals a day, we spend most of our daily life in a postprandial state. It is therefore important that we thoroughly understand postprandial changes and subsequent effects on cardiometabolic health.

Postprandial lipemia

Elevated and prolonged postprandial lipemia – as indicated by increased serum triacylglycerol concentrations – is associated with an increased risk of the development of CHD [31]. After intake of a single, fat-containing meal, triacylglycerol concentrations (which are mainly present in chylomicrons) normally peak after 3 to 5 hours and the duration of postprandial lipemia is about 6 to 8 hours in healthy people [32]. The peak concentration of triacylglycerols as well as the duration of lipemia depends on the amount and type of fat present in the meal. It has been shown that more than 15 grams of fat is needed to induce a triacylglycerol response and that intakes of 30, 40 or 50 grams of fat result in a stepwise increase in postprandial lipemia [33]. In addition, the intake of multiple fat-containing meals enhances lipemia [34]. Since people generally consume multiple meals a day, it is of interest to examine consecutive meal challenges to more closely mimic daily-life. Fatty-acid composition of the meal has less clear effects on triacylglycerol responses, but there are indications that SFA and MUFA have comparable effects on postprandial triacylglycerol concentrations, while n-6 and n-3 PUFAs tend to lower lipemia as compared to other fatty acids [35, 36]. In addition, the number and type of chylomicrons carrying the triacylglycerols (e.g. chylomicron size and/or apolipoprotein content) seems to depend on the fatty-acid composition of the meal [36], which shows the complexity of postprandial lipid metabolism. In addition, the physical characteristics of a fat – in particular the amount of solid fat at 37°C – affect postprandial lipid response independent of the fatty-acid composition [37]. Lastly, postprandial lipemia is also influenced by many factors related to the individual, such as age, gender, obesity, type 2 diabetes, and genetics [31]. Increasing age is associated with higher postprandial triacylglycerol concentrations and women generally have lower postprandial lipemia than men. In addition, postmenopausal women have higher postprandial triacylglycerol concentrations than premenopausal women. Under pathological conditions – both of metabolic and genetic origin – such as obesity, type 2 diabetes or familial hypercholesterolemia, peak concentrations can be 2 to 3 times higher and the duration of lipemia can be prolonged up to 10 to 12 hours after meal in take [31, 32].

Postprandial glycemia

Postprandial glycemia – increased plasma glucose concentrations after consumption of a meal – is also associated with CVD [38]. In contrast to triacylglycerol concentrations, glucose concentrations rise quickly after meal intake and generally peak within the first hour. For example, in healthy young adults without overweight, the average time for glucose

concentrations to peak was between 46 to 50 minutes after multiple meal intakes during the day [39]. How long glucose concentrations remain elevated also depends on the sensitivity of the individual to insulin. Postprandial hyperglycemia means that glucose peak concentrations are elevated, which can for example be assessed by a glucose tolerance test, i.e. glucose concentrations ≥ 7.8 mmol/L 2 hours after intake of 75 grams of glucose [40]. In 1981, Jenkins et al. introduced the glycemic index which classifies a food based on its effect on postprandial glucose concentrations [41]. Besides the amount and type of carbohydrates, also the presence of fat and protein in a meal affects the glycemic index and thus postprandial glycemia [42, 43]. Consuming fat and/or protein together with carbohydrates lowers postprandial glycemia by slowing down gastric emptying or by increasing insulin secretion. Exact mechanisms are however not yet fully understood, and the effects of fat and protein are likely mediated via different mechanisms. Not much is known about effects of different dietary fatty acids on postprandial glucose metabolism, but one study has reported that meals rich in MUFA or PUFAs lower postprandial glycemia compared with meals rich in SFA [44]. Like for postprandial lipemia, also factors related to the individual affect postprandial glucose concentrations such as BMI and age. Postprandial glucose concentrations are for example higher in subjects with obesity (BMI ≥ 30 kg/m^2) compared with subjects with a healthy-weight (BMI < 25 kg/m^2) [45]. Also increasing age is associated with higher postprandial glycemia [46]. Besides, the so-called 2nd meal effect is a well-known phenomenon for postprandial glucose responses, i.e. the response after a second meal depends on the composition of the first meal [47]. For instance, a breakfast with a low glycemic index lowers postprandial glycemia after lunch compared with a breakfast with a high glycemic index [48] and this second-meal effect can even last overnight, i.e. the glycemic index of a dinner affects the glucose response after breakfast the following morning [49].

Postprandial HDL-mediated cholesterol efflux capacity

In addition to lipemia and glycemia, postprandial HDL-mediated cholesterol efflux may also be important with respect to atherosclerosis. Not much is known yet about effects of different macronutrients on postprandial CEC, but it has been shown that consumption of a high-fat meal increased CEC up to 8 hours after meal intake compared to fasting concentrations [50-53]. If carbohydrates and proteins also affect postprandial cholesterol efflux is currently unknown. Moreover, the mechanism underlying postprandial changes in CEC after a high-fat meal is not well understood. Possibly, postprandial lipemia influences HDL functionality via modifying particle characteristics [51]. By comparing the effects of the different macronutrients on postprandial HDL-mediated cholesterol efflux, we get more insight in underlying mechanisms, i.e. if changes in postprandial HDL-mediated CEC are related to changes in triacylglycerols (induced by a high-fat meal), glucose and insulin (high-carbohydrate meal), or insulin (high-protein meal). In addition, postprandial changes in the metabolism of apoA-I – the protein on HDL particles responsible for ABCA1-mediated

cholesterol efflux – may be related to changes in postprandial HDL-mediated cholesterol efflux.

Recommended intakes of dietary fatty acids are mainly based on their effects on fasted serum LDL-cholesterol concentrations, even though evidence for a role of postprandial metabolism on CHD-risk is growing. If palmitic acid and stearic acid differently affect postprandial lipemia, glycemia, and HDL-mediated cholesterol efflux is not clear.

This dissertation

The aim of this dissertation was to study effects of dietary fat – predominantly the different saturated fatty acids palmitic acid and stearic acid –, carbohydrates, and proteins on fasting and postprandial HDL-mediated cholesterol efflux via the ABCA1-pathway and on other markers relevant for cardiometabolic diseases. For this, we have reviewed the existing literature on effects of palmitic acid and stearic acid on cardiometabolic risk markers in chapter 2. In addition, a human intervention study has been performed in which we have examined longer-term and postprandial effects of palmitic-acid versus stearic-acid intakes on HDL-mediated cholesterol efflux via ABCA1 as well as other cardiometabolic risk markers. Results of this study on fasting cardiometabolic risk markers are reported in chapter 3, results on postprandial lipemia and glycemia in chapter 4, and results on fasting lipoprotein subfractions as well as fasting and postprandial cholesterol efflux and secretion of apoA-I in chapter 5. Lastly, we have studied in humans the effects on postprandial HDL-mediated cholesterol efflux via ABCA1 and secretion of apoA-I after acute intakes of fats, carbohydrates, or proteins, and results are reported in chapter 6.

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CHAPTER 2

Palmitic acid versus stearic acid: effects of interesterification and intakes on cardiometabolic risk markers – a systematic review

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Abstract

Background: Fats rich in palmitic or stearic acids can be interesterified to increase their applicability for the production of certain foods. Compared with palmitic acid, stearic acid lowers low-density lipoprotein (LDL)-cholesterol, a well-known risk factor for coronary heart disease (CHD), but effects on other cardiometabolic risk markers have been studied less extensively. In addition, the positional distribution of these two fatty acids within the triacylglycerol molecule may affect their metabolic effects.

Objective: The objective was to compare the longer-term and postprandial effects of (interesterified) fats rich in either palmitic or stearic acids on cardiometabolic risk markers in humans.

Methods: Two searches in PubMed/Medline, Embase (OVID) and Cochrane Library were performed; one to identify articles that studied effects of the position of palmitic or stearic acids within the triacylglycerol molecule, and one to identify articles that compared side-by-side effects of palmitic acid with those of stearic acid.

Results and Conclusions: Interesterification of palmitic acid- or stearic acid-rich fats does not seem to affect fasting serum lipids and (apo)lipoproteins. However, substituting palmitic acid with stearic acid lowers LDL-cholesterol concentrations. Postprandial lipemia is attenuated if the solid fat content of a fat blend at body temperature is increased. How (interesterification of) palmitic or stearic acid-rich fats affects other cardiometabolic risk markers needs further investigation.

Introduction

During the last decades, many studies have been carried out to gain more insight into the effects of dietary fat intake on risk markers for cardiovascular disease (CVD) such as disturbances in lipid metabolism, glucose-insulin homeostasis, the haemostatic system, or low-grade systemic inflammation. A well-accepted risk factor for coronary heart disease (CHD) is low-density lipoprotein (LDL)-cholesterol (LDL-C), which is increased by diets rich in saturated and trans fatty acids. Guidelines to prevent CHD are therefore focused on the exchange of dietary saturated and trans fats for unsaturated fats [1]. Saturated fat, however, is a collective term for different saturated fatty acids that exert different metabolic effects. In the Western diet, palmitic acid (C16:0) and stearic acid (C18:0) are the most commonly consumed saturated fatty acids [2]. It is generally believed that palmitic acid is more cholesterol-raising than stearic acid [3, 4]. However, the effects of palmitic and stearic acids on other cardiometabolic risk markers are less well established. Besides chain length of saturated fatty acids, also the positional distribution of fatty acids within the triacylglycerol (TAG) molecule may be important for their metabolic effects [5]. TAG molecules consist of a glycerol backbone to which three fatty acids are esterified. The positional distribution of these fatty acids within the TAG molecule, the so-called TAG structure, can be specified by stereospecific numbering (sn) as sn-1, sn-2, and sn-3. With interesterification, a chemical or enzymatic process used by the food industry, fatty acid positions can be exchanged within and between TAG molecules, thereby creating new TAG structures. This structure determines the physical properties of a fat including its melting behavior which in turn determines the suitability of the fat for the food industry; solid fats are for instance more suitable for baked goods and certain types of margarines than oils. Some vegetable oils such as palm oil contain relatively high amounts of palmitic and/or stearic acid predominantly at the outer sn-1 and -3 positions [6]. Interesterification of these oils increases the amounts of palmitic or stearic acids at sn-2, which will increase their melting points. Since no trans fatty acids are generated by interesterification, this process seems to be a good alternative for partially hydrogenated trans fats. However, the positional distribution of fatty acids may affect their metabolic fate, also because the dietary fatty acid at the sn-2 position is largely retained when incorporated into chylomicron TAG molecules [7]. Given that fats rich in palmitic and/or stearic acid are often used for interesterification, it is important that we thoroughly understand their impact on metabolic health. We have therefore systematically reviewed the current knowledge on the longer-term and postprandial effects on cardiometabolic risk markers of 1) the effect of interesterification of either palmitic acid- or stearic acid-rich fats and 2) the difference between palmitic acid- and stearic acid-rich fats.

Methods

The databases PubMed/Medline, Embase (OVID) and Cochrane Library were searched for papers published until December 2019. Two searches were performed; one to identify articles that studied effects of the position of palmitic acid or stearic acid on the TAG molecule, and one to identify articles that compared side-by-side effects of palmitic acid with those of stearic acid. For the effect of TAG structure, the following search strategies were used: ((interesterified[All Fields] OR "esterification"[MeSH Terms] OR "TAG structures"[All Fields] OR "triglycerides/administration and dosage"[MeSH Terms]) AND ("palmitic acid"[All Fields] OR "stearic acid"[All Fields])) for PubMed, ((triglyceride structure/ OR *triacylglycerol/ OR interesterification.mp.) AND (stearic acid/ OR palmitic acid/)) with 'article' as filter for Embase, and ((esterification [MeSH descriptor] OR triglycerides [MeSH descriptor with qualifier administration and dosage] OR TAG structures OR interesterified) AND (palmitic acid OR stearic acid)) in Cochrane Library. For the comparison of palmitic acid with stearic acid, the following search strategies were used: (("palmitic acid"[All Fields] OR "palmitate"[All Fields] OR "hexadecanoic acid"[All Fields] OR "C16:0"[All Fields]) AND ("stearic acid"[All Fields] OR "octadecanoic acid"[All Fields] OR "stearate"[All Fields] OR "C18:0"[All Fields])) AND "clinical study"[Publication Type] for Pubmed, (*palmitic acid/ and *stearic acid/ and human.mp) for Embase, and (palmitic acid AND stearic acid) for Cochrane Library.

Studies were eligible if they met the following inclusion criteria: human dietary intervention trial comparing diets or meals containing either palmitic or stearic acid mainly at sn-1 and -3 with diets or meals containing higher amounts of palmitic or stearic acid at the sn-2 position or comparing diets or meals rich in palmitic acid with diets or meals rich in stearic acid; diets or meals had comparable contents of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs); subjects were ≥ 18 years and apparently healthy; cardiometabolic risk markers (lipids and lipoproteins, hematological markers, glucose-insulin homeostasis, endothelial function markers, and/or inflammation markers) were assessed; articles were published in English and available as full text.

The search for the effect of the position of either palmitic or stearic acid within the TAG molecule resulted in a total of 932 records (248 from PubMed, 646 from Embase, 38 from Cochrane) of which 100 records were duplicates. Twenty-six records from the remaining 832 were considered to be of interest based on their titles and abstracts. After screening of the full texts, two articles were excluded because the fatty-acid contents of the experimental fats were not comparable, one because no cardiometabolic risk markers were assessed, one because subjects had type 2 diabetes, and five because they were conference abstracts. Reference lists of all eligible papers were searched for additional studies, which resulted in another three articles. In the end, a total of 20 articles corresponding to 19 human intervention trials were included (**Supplemental Figure 1**).

The search for palmitic acid versus stearic acid resulted in a total of 372 records (111 from PubMed/Medline, 125 from Embase and 136 from Cochrane) of which 97 records were duplicates. Twenty-four records from the remaining 275 were considered to be of interest based on their titles and abstracts. After screening of the full texts, two articles were excluded because experimental fats differed not only in palmitic acid and stearic acid contents but also in other fatty acids and four other articles because they were conference abstracts. Reference lists of all eligible papers and previous reviews were searched for additional studies, which resulted in another four articles. In the end, a total of 22 articles corresponding to 17 human trials were included (Supplemental Figure 2).

Results

Longer-term effects of sn-2 content of palmitic acid or stearic acid on fasting cardiometabolic risk markers

Six studies have compared side-by-side the effects on fasting cardiometabolic risk markers of diets with high versus low proportions of palmitic acid at the sn-2 position (Supplemental Table 1) and two studies with high versus low proportions of stearic acid at sn-2 (Supplemental Table 2). Results are summarized in Table 1. In seven studies, the content of palmitic or stearic acid at sn-2 was increased by interesterification of the experimental fats, while in one study interesterification decreased the sn-2 content of palmitic acid [8]. Studies examining palmitic acid-rich fats used palm oil [9, 10], palm olein [11, 12], butter [8], or a blend consisting mainly of coconut and palm oil [13]. Two studies have reported the solid fat content at 37°C; in one study both the native and interesterified palm oils were liquid [9], while in the other study interesterification increased the solid fat content of palm olein from 0 to 6% [11]. Sources for the stearic acid-rich fats were shea butter [14] and cocoa butter [15]. Interesterification of shea butter increased the solid fat content at 37°C from 22 to 41% [14]. The melting points of native and interesterified cocoa butter were not measured, but the authors indicated that native cocoa butter was liquid at 37°C and assumed that the solid fat content of the interesterified fat at 40.5°C was 19% [15]. Most studies had used a randomized cross-over design, except for two studies that used a parallel design [8, 12]. Experimental periods varied from 21 to 56 days for studies examining palmitic acid-rich fats and diets provided 1 to 11 energy percent (en%) of palmitic acid. The proportion of palmitic acids at sn-2 was reported in five out of seven studies and differed between 11 and 60% of total fatty acids. The two studies examining stearic acid-rich fats had interventions periods of 18 and 21 days, and diets provided 10 and 7 en% stearic acid. One study reported proportions of stearic acid at sn-2, and the difference between diets was approximately 20%.

Lipids and (apo)lipoproteins

Interesterification of palmitic acid-rich fats did not affect concentrations of TAG, total cholesterol (TC), LDL-C, or high-density lipoprotein (HDL)-C [8-13]. However, one study reported that men - but not women - showed a small, but statistically significant increase in TC and LDL-C concentrations in response to the diet with a higher sn-2 content of palmitic acid [9]. No differences were found for non-esterified fatty acid (NEFA) [13], apolipoprotein (apo)B [8, 12], apoA1 [8, 11, 12], and lipoprotein[a] concentrations [11-13]. The sn-2 content of stearic acid also had no effects on concentrations of TAG [14, 15], TC [14, 15], LDL-C, or HDL-C [14].

Hematological markers

Only two studies have examined the effects of interesterification on hematological markers. No effects were found of sn-2 content of palmitic acid on concentrations of activated form of coagulation factor VII (FVIIa), fibrinogen, plasminogen activator inhibitor (PAI)-1 antigen, tissue plasminogen activator (tPA) antigen and its activity, and von Willebrand factor (vWF) [13], and of stearic acid sn-2 content on FVIIa concentrations [14].

Other markers

The proportion of palmitic acids at sn-2 did not affect concentrations of glucose [11-13], insulin [11, 12], C-peptide [11, 12], and C-reactive protein (CRP) [13]. Stearic acid sn-2 content also did not affect glucose and insulin concentrations [14].

Longer-term effects of substituting palmitic acid with stearic acid on fasting cardiometabolic risk markers

Eleven studies have compared side-by-side the effects of diets rich in palmitic acid with those of diets rich in stearic acid on fasting cardiometabolic risk markers (Table 2 and Supplemental Table 3). The palmitic acid sources used were palm oil [15-20], (interesterified) palm olein [21, 22], a blend containing tripalmitin [23], and palm stearin [22]. For stearic acid-rich diets, cocoa butter [15, 19, 20, 24], hydrogenated soybean oil [16, 21], shea butter [17, 18], hydrogenated canola [22], a blend containing tristearin [23], and an interesterified blend containing fully hydrogenated soybean oil [12] were used. Except for one study [12], all studies used a randomized cross-over design. Experimental periods varied from 18 to 56 days and diets provided 4 to 18 en% from palmitic acids or stearic acids. Exchange of palmitic acids with stearic acids between the diets varied between 1 and 15 en%.

Table 1. Summary of studies examining the longer-term effects of substituting fats low in palmitic acid (C16:0) or stearic acid (C18:0) sn-2 contents with fats high in C16:0 or C18:0 sn-2 contents resp.

Fasted Lipids and lipoproteins	High vs low C16:0 sn-2	High vs low C18:0 sn-2	Hemato-logical markers	High vs low C16:0 sn-2	High vs low C18:0 sn-2	Other markers	High vs low C16:0 sn-2	High vs low C18:0 sn-2
TAG	0 ↓ 6 = 0 ↑	0 ↓ 2 = 0 ↑	FVIIa	0 ↓ 1 = 0 ↑	0 ↓ 1 = 0 ↑	Glucose	0 ↓ 3 = 0 ↑	0 ↓ 1 = 0 ↑
NEFA	0 ↓ 1 = 0 ↑	NA	Fibrino-gen	0 ↓ 1 = 0 ↑	NA	Insulin	0 ↓ 2 = 0 ↑	0 ↓ 1 = 0 ↑
TC	0 ↓ 6 =* 0 ↑	0 ↓ 2 = 0 ↑	PAI-1	0 ↓ 1 = 0 ↑	NA	C-peptide	0 ↓ 2 = 0 ↑	NA
LDL-C	0 ↓ 6 =* 0 ↑	0 ↓ 1 = 0 ↑	tPA	0 ↓ 1 = 0 ↑	NA	CRP	0 ↓ 1 = 0 ↑	NA
HDL-C	0 ↓ 6 = 0 ↑	0 ↓ 1 = 0 ↑	vWF	0 ↓ 1 = 0 ↑	NA			
ApoB	0 ↓ 3 = 0 ↑	NA						
ApoA1	0 ↓ 3 = 0 ↑	NA						
Lp[a]	0 ↓ 3 = 0 ↑	NA						

Markers are significantly lower (↓), higher (↑) or not significantly different (=) after intake of fats high in C16:0 sn-2 or C18:0 sn-2 contents compared with fats low in C16:0 sn-2 or C18:0 sn-2 contents respectively. *=In men, total and LDL cholesterol concentrations were slightly increased (0.10 mmol/L and 0.08 mmol/L respectively) on the diet with higher C16:0 sn-2 [9]. Abbreviations: apoB, apolipoprotein B; apoA1, apolipoprotein A1; CRP, C-reactive protein; FVIIa, activated factor VII; HDL-C, high-density lipoprotein cholesterol; Lp[a], lipoprotein [a]; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; PAI, plasminogen activator inhibitor; TAG, triacylglycerol; TC, total cholesterol; tPA, tissue plasminogen activator; vWF, von Willebrand Factor.

Lipids and (apo)lipoproteins

Concentrations of TAG did not differ between the diets [15-24], except in one study where TAG concentrations were lower after an interestified stearic acid-rich diet [12]. However, the majority of studies found lower TC concentrations on the stearic acid-rich diet compared with palmitic acid [15-20, 23]. In five of these studies, LDL-C concentrations were also decreased [16-18, 20, 23], and in two studies the concentration of LDL-C tended to be lower on stearic acid [12, 19]. Lower HDL-C concentrations on the stearic acid-rich diet were found in three studies [17, 19, 20], while in seven other studies no significant differences were found [12, 16, 18, 21-24]. No changes in concentrations of VLDL-C were reported [17, 19, 20]. Of the studies that measured apoB and apoA1 [12, 17, 19, 23], one observed decreased concentrations of apoB [17] and two of apoA1 [17, 19] on the stearic acid-rich diet. Lipoprotein[a] concentrations were were higher on the stearic acid-rich diet in one study [25] but no differences were observed in another study [12].

Hematological markers

One study found decreased factor VII coagulant activity (FVIIc) on the stearic acid-rich diet compared with palmitic acid [17]. However, FVIIc activities were not different between diets in another study [22]. Mean platelet volume (MPV) was lower in one study [22], but no difference was observed in another study of the same group [24]. No differences between diets were reported for other hematological markers [17, 20, 22, 24]. In one study, various inflammation markers were measured and no significant differences were observed [20].

Other markers

Stearic acid decreased cholesteryl ester transfer protein (CETP) activity compared with palmitic acid in one study [19] and a similar decrease was observed in another study although not significant [23]. No effects on lecithin-cholesterol acyltransferase (LCAT) activity were observed [23]. Three studies examined effects on glucose metabolism. An intravenous glucose tolerance test was performed and a comparable response in glucose and insulin was observed on both diets [26]. No differences were observed in fasting concentrations of glucose [12, 20], insulin [12, 20], and C-peptide [12].

Table 2. Summary of studies examining the longer-term effects of substituting fats high in palmitic acid (C16:0) with fats high in stearic acid (C18:0).

Fasted	C18:0 vs C16:0		C18:0 vs C16:0		C18:0 vs C16:0		C18:0 vs C16:0
Lipids and lipoproteins		Hemato-logical markers				Other markers	
TAG	1↓ 10 = 0↑	FVIIc	1↓ 1 = 0↑	Fibrino-gen	0↓ 1 = 0↑	CETP activity	1↓ 1 = 0↑
TC	7↓ 4 = 0↑	MPV	1↓ 1 = 0↑	Plasmino-gen	0↓ 1 = 0↑	LCAT activity	0↓ 1 = 0↑
VLDL-C	0↓ 4 = 0↑	PAI-1 activity	0↓ 1 = 0↑	WBC	0↓ 2 = 0↑	Glucose	0↓ 2 = 0↑
LDL-C	5↓ 5 = 0↑	PAI-1 antigen	0↓ 1 = 0↑	RBC	0↓ 2 = 0↑	Insulin	0↓ 2 = 0↑
HDL-C	3↓ 7 = 0↑	tPA activity	0↓ 1 = 0↑	Hb	0↓ 2 = 0↑	C-peptide	0↓ 1 = 0↑
ApoB	1↓ 4 = 0↑	tPA antigen	0↓ 1 = 0↑	PLT	0↓ 2 = 0↑	Various inflammation markers	0↓ 1 = 0↑
ApoA1	2↓ 3 = 0↑	EFA	0↓ 1 = 0↑	APTT	0↓ 1 = 0↑		
Lp[a]	0↓ 1 = 1↑	Thrombo modulin	0↓ 1 = 0↑	ATIII	0↓ 1 = 0↑		
		PT	0↓ 1 = 0↑	PTT	0↓ 1 = 0↑		

Markers are significantly lower (↓), higher (↑) or not significantly different (=) after intake of fats high in C18:0 compared with fats high in C16:0. Abbreviations: apoB, apolipoprotein B; apoA1, apolipoprotein A1; APTT, activated partial thromboplastin time; ATIII, antithrombin III; CETP, cholesteryl ester transfer protein; EFA, euglobulin fibrinolytic activity; FVIIc, Factor VII coagulant activity; HDL-C, high-density lipoprotein cholesterol; Hb, hemoglobin; IE, interesterified; LCAT, lecithin-cholesterol acyltransferase; Lp[a], lipoprotein [a]; LDL-C, low-density lipoprotein cholesterol; MPV, mean platelet volume; PAI, plasminogen activator inhibitor; PLT, platelet count; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cells; TAG, triacylglycerol; TC, total cholesterol; tPA, tissue plasminogen activator; VLDL, very low-density lipoprotein; WBC, white blood cells.

Postprandial effects of sn-2 content of palmitic acid or stearic acid on cardiometabolic risk markers

Eight studies have compared side-by-side postprandial effects of meals with high versus low proportions of palmitic acid at the sn-2 position (**Supplemental Table 4**), and four studies with high versus low proportions of stearic acid (**Supplemental Table 5**). Results are summarized in **Table 3**. Most of the studies examining palmitic acid-rich meals have used palm olein. Interesterification of palm olein not only increased the palmitic acid content at sn-2, but also the solid fat content at 37°C. In one study, lard was used [27], in which interesterification decreased the palmitic acid at sn-2 as well as the solid fat content. Another study used a commonly consumed blend of palm stearin and palm kernel (PSt/PK) [28]. Interesterification of the PSt/PK blend increased palmitic acid at sn-2, but decreased the solid fat content at 37°C. The stearic acid-rich meals consisted of structured TAG molecules with predominantly stearic and oleic acid (C18:1) [29], cocoa butter [30], shea butter [14], or canola stearin [31]. Interesterification of cocoa and shea butter increased the proportion of stearic acid at sn-2 and the solid fat content at 37°C, which decreased after interesterification of canola stearin. For palmitic acid-rich meals, total fat content of the meals varied between 40 and 75 grams, of which 12 to 30 grams originated from palmitic acid. Differences between meals in the proportion of palmitic acids at sn-2 varied between 17.0 and 66.8% of total fatty acids at sn-2. For stearic acid-rich meals, total fat content varied between 50 and 102 grams including 17 to 30 grams of stearic acid. Two of the four studies reported the proportions of stearic acids at sn-2 and differences between meals were 19.7 and 25.0%. Postprandial follow-up varied between 4 and 8 hours.

Lipids and (apo)lipoproteins

A lower postprandial TAG response - as indicated by the incremental area under the curve (iAUC) - was observed in one study after a meal with higher palmitic acid sn-2 content [32]. The same tendency was found in three other studies [27, 33, 34], and in one study this was accompanied by a significant lower response in the first four hours after the meal with a higher proportion of palmitic acid at sn-2 [34]. In contrast, one study showed an increased TAG response after a higher palmitic acid sn-2 content [28]. Two other studies found no differences in TAG responses [35, 36]. Postprandial responses of NEFAs [7, 27, 32, 34-36], TC [7, 27, 33, 34], and HDL-, LDL- [33], VLDL-, and chylomicron cholesterol [27, 32] were comparable. ApoB48 responses were measured in one study and no effect of sn-2 palmitic acid content was observed [7]. For stearic acid, three studies found no changes in total TAG responses in healthy-weight subjects [14, 29, 31]. In one of these studies an obese group was included, in which the TAG response was decreased after the high sn-2 stearic acid meal [31]. In addition, in another study, higher sn-2 stearic acid content decreased the TAG response in healthy-weight subjects [30]. NEFA responses were not differently affected [14, 29, 31]. In addition,

responses of TC as well as of LDL-C and HDL-C were comparable between meals that differed in stearic acid sn-2 content [14, 30, 31].

Hematological markers

In one study, no effect of palmitic acid sn-2 content was observed on FVIIa responses [33]. Interestingly, the effects of stearic acid sn-2 content were different between fat sources, i.e. cocoa butter with a lower stearic acid content at sn-2 increased FVIIa postprandial compared with cocoa butter with a higher sn-2 content [30], while the amount of stearic acid at the sn-2 position of shea blends had no effect on FVIIa [14].

Other markers

Postprandial glucose and insulin responses after palmitic acid-rich meals were comparable [27, 28, 32, 33, 35-37]. However, one study found that the peak value of insulin appeared faster after the meal with higher sn-2 content of palmitic acid (after 60 instead of 90 minutes) [32], while another study observed lower insulin concentrations 30, 90 and 120 minutes after intake of the high sn-2 meal which was accompanied by a tendency towards a lower total insulin response [33]. Furthermore, one study found lower glucose-dependent insulinotropic polypeptide (GIP) concentrations after the high sn-2 meal [37], while two other studies did not observe any differences [28, 35]. Two studies also measured peptide YY (PYY) and no significant differences were found [28, 37], although in one study PYY response tended to be less in women [37]. Only one study examined inflammatory cytokines and the endothelial function marker E-selectin, and no differences were found [7]. Three studies examining stearic acid-rich meals measured postprandial glucose and insulin, and responses were comparable between the meals [14, 29, 31]. Furthermore, white blood count (WBC), as measured in one study, was not affected [14].

Table 3. Summary of studies examining the postprandial effects of substituting fats low in sn-2 palmitic acid (C16:0) or stearic acid (C18:0) contents with fats high in sn-2 C16:0 or C18:0 contents respectively.

Postprandial Lipids and lipoproteins	High vs low C16:0 sn-2	High vs low C18:0 sn-2	Hemato-logical markers	High vs low C16:0 sn-2	High vs low C18:0 sn-2	Other markers	High vs low C16:0 sn-2	High vs low C18:0 sn-2
TAG	1 ↓ 6 = 1 ↑	2 ↓ 3 = 0 ↑	FVIIa	0 ↓ 1 = 0 ↑	1 ↓ 1 = 0 ↑	Glucose	0 ↓ 7 = 0 ↑	0 ↓ 3 = 0 ↑
NEFA	0 ↓ 6 = 0 ↑	0 ↓ 3 = 0 ↑	WBC	0 ↓ 1 = 0 ↑	0 ↓ 1 = 0 ↑	Insulin	0 ↓ 7 = 0 ↑	0 ↓ 3 = 0 ↑
TC	0 ↓ 4 = 0 ↑	0 ↓ 3 = 0 ↑				C-peptide	0 ↓ 1 = 0 ↑	NA
VLDL-C	0 ↓ 2 = 0 ↑	NA				GIP	1 ↓ 2 = 0 ↑	NA
LDL-C	0 ↓ 1 = 0 ↑	0 ↓ 3 = 0 ↑				PYY	0 ↓ 2 = 0 ↑	NA
HDL-C	0 ↓ 1 = 0 ↑	0 ↓ 2 = 0 ↑				IL-6	0 ↓ 1 = 0 ↑	NA
CM-C	0 ↓ 2 = 0 ↑	NA				IL-8	0 ↓ 1 = 0 ↑	NA
ApoB48	0 ↓ 1 = 0 ↑	NA				TNF-α	0 ↓ 1 = 0 ↑	NA
						E-selectin	0 ↓ 1 = 0 ↑	NA

Markers are significantly lower (↓), higher (↑) or not significantly different (=) after intake of fats high in C16:0 sn-2 or C18:0 sn-2 contents compared with fats low in C16:0 sn-2 or C18:0 sn-2 contents respectively. Abbreviations: apoB48, apolipoprotein B48; CM-C, chylomicron cholesterol; FVIIa, activated factor VII; GIP, glucose-dependent insulinotropic polypeptide; HDL-C, high-density lipoprotein cholesterol; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; PYY, peptide YY; TAG, triacylglycerol; TC, total cholesterol; TNF, tumor necrosis factor; VLDL, very low-density lipoprotein; WBC, white blood cells.

Postprandial effects of substituting palmitic acid with stearic acid on cardiometabolic risk markers

Six studies have compared side-by-side postprandial effects of meals high in palmitic acid with those high in stearic acid (**Table 4**). The fats added to enrich meals with palmitic acid were palm oil [38, 39], palm olein [7, 40], and a blend of tripalmitin with high-oleic sunflower oil (HOSO) [41]. For the stearic acid-rich meals, lard [7, 38, 40], hydrogenated HOSO [39], and a blend of tristearin with HOSO [41] were used. Fat content of the test meals varied between 50 and 90 grams, from which 9 to 37 grams originated from palmitic or stearic acids. Difference between palmitic and stearic acid in the meals ranged between 5 and 23 en%. Postprandial follow-up varied between 4 and 8 hours (**Supplemental Table 6**).

Lipids and (apo)lipoproteins

In two studies, a lower TAG response after the meal rich in stearic acid was observed [7, 40] and in another study lower TAG concentration 3 hours after the stearic acid-rich meal [39]. Other studies did not observe any differences [38, 41, 42]. The postprandial reduction in NEFAs was lower after stearic-acid intake in one study [7] but no differences were observed between meals in two other studies [40, 41]. Postprandial responses of VLDL-C, LDL-C, HDL-C, apoB, and apoA1 were measured in one study, but did not differ over time and between meals [41]. Also, the responses in postprandial concentrations of lipoprotein[a] [43], TC and apoB48 [7] were not differently affected.

Hematological markers

Postprandial responses of FVIIa after a meal rich in palmitic or stearic acid were comparable [39, 42, 44]. However, one study observed a non-significant lower response of FVIIa 2 to 6 hours after the stearic acid-rich meal with relatively stable FVIIa concentrations between 4 and 8 hours, while FVIIa peaked 6 hours after palmitic acid and then declined [44]. FVIIc responses were measured in two studies. In one study, no differences between the meals were found [39]. In the other study, however, 8 hours after the palmitic acid-rich meal FVIIc had almost returned to baseline, while it reached its highest value 8 hours after the stearic acid-rich meal. Nevertheless, no difference was found in total FVIIc response [44].

Other markers

Postprandial responses of glucose [37, 40], insulin [37, 38, 40], and C-peptide [37] were not differently affected. However, secretion of GIP was lower after intake of stearic acid-rich lard [37]. Postprandial changes in concentrations of leptin [38, 40], inflammatory cytokines [7, 40], E-selectin [7], and PYY [37] were comparable. In addition, changes in CETP and lipoprotein lipase (LPL) activity did not differ between meals [41].

Table 4. Summary of studies examining the postprandial effects of substituting fats high in palmitic acid (C16:0) with fats high in stearic acid (C18:0).

Postprandial Lipids and lipoproteins	C18:0 vs C16:0	Hemato-logical markers	C18:0 vs C16:0	Other markers	C18:0 vs C16:0
TAG	1 ↓ 4 = 0 ↑	FVIIa	0 ↓ 3 = 0 ↑	Glucose	0 ↓ 1 = 0 ↑
NEFA	0 ↓ 2 = 0 ↑	FVIIc	0 ↓ 2 = 0 ↑	Insulin	0 ↓ 2 = 0 ↑
TC	0 ↓ 1 = 0 ↑	PAI-1 antigen	0 ↓ 1 = 0 ↑	GIP	1 ↓ 0 = 0 ↑
VLDL-C	0 ↓ 1 = 0 ↑	tPA activity	0 ↓ 1 = 0 ↑	PYY	0 ↓ 1 = 0 ↑
LDL-C	0 ↓ 1 = 0 ↑			Leptin	0 ↓ 1 = 0 ↑
HDL-C	0 ↓ 1 = 0 ↑			CETP activity	0 ↓ 1 = 0 ↑
ApoB	0 ↓ 1 = 0 ↑			LPL activity	0 ↓ 1 = 0 ↑
ApoA1	0 ↓ 1 = 0 ↑			IL-6	0 ↓ 1 = 0 ↑
Lp[a]	0 ↓ 1 = 0 ↑			TNF-α	0 ↓ 1 = 0 ↑
				IL-1β	0 ↓ 1 = 0 ↑

Markers are significantly lower (↓), higher (↑) or not significantly different (=) after intake of fats high in C18:0 compared with fats high in C16:0. Abbreviations: apoB, apolipoprotein B; apoA1, apolipoprotein A1; CETP, cholesteryl ester transfer protein; FVIIa, activated factor VII; FVIIc, Factor VII coagulant activity; GIP, glucose-dependent insulinotropic polypeptide; HDL-C, high-density lipoprotein cholesterol; IL, interleukin; Lp[a], lipoprotein [a]; LPL, lipoprotein lipase; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; PAI, plasminogen activator inhibitor; PYY, peptide YY; TAG, triacylglycerol; TC, total cholesterol; TNF, tumour necrosis factor; tPA, tissue plasminogen activator; VLDL, very low-density lipoprotein.

Discussion

Interesterification is widely used by the food industry to modify TAG structures of fats to change their physical characteristics and thereby increase their suitability for food applications without the formation of trans fatty acids. The saturated fatty acids within interesterified fats are predominantly palmitic acid and stearic acid. To better understand metabolic effects of interesterified fats we have systematically reviewed effects of fats rich in either palmitic or stearic acid on cardiometabolic risk markers. Focus was on the position of palmitic acid or stearic acid within the TAG molecule and on studies that have compared side-by-side palmitic acid- versus stearic acid-rich fats.

Longer-term effects

Although exact intakes of interesterified fats are unknown, it has been estimated that – if all trans fats would be replaced with interesterified fats – the mean daily intake in the United States would be approximately 3 en% with an upper limit of 4.8 en% [45]. Daily intakes of interesterified fats as well as the proportions of total and sn-2 palmitic or stearic acids differed widely between studies. However, in most studies, interesterified fat intakes were well above the estimated upper limit of 4.8 en% [45]. Still, no effects of palmitic acid or stearic acid sn-2 content were found. In general, metabolically healthy and relatively young subjects were studied. In the only study that included mildly hypercholesterolemic subjects, also no effects of palmitic acid sn-2 content were observed [10]. Furthermore, studies using stearic acid-rich fats have been performed in men only. It is known that men and women differ in CVD risk [46] and may respond differently to dietary interventions [47]. Indeed, one study observed slightly increased TC and LDL-C in men but not in women after intake of a fat with a higher palmitic acid sn-2 content [9]. The difference between men and women was however not statistically significant, but this may be explained by lack of statistical power. Little research has been done on the hemostatic system, inflammation, and glucose-insulin homeostasis, which are all involved in the pathogenesis of CVD [48-50]. However, results so far do not indicate effects of diets enriched with interesterified fats on markers involved in these metabolic processes.

Since the use of interesterified fats may increase stearic and/or palmitic acid intakes, we need to thoroughly understand their metabolic effects. Daily intakes of palmitic and stearic acids in the United States are approximately 6 en% and 3 en% respectively [51]. It is well known that stearic acid lowers concentrations of TC, LDL-C, and HDL-C as compared with palmitic acid [52]. Indeed, the majority of studies showed decreased TC and LDL-C concentrations on the stearic acid-rich diet [16-18, 23]. In three studies, lower HDL-C concentrations were observed [17, 19, 20]. Only one out of four studies observed a statistically significant decrease in apoB100 concentrations on the stearic acid-rich diet [17]. However, previous meta-analyses found lower apoB concentrations on stearic acid compared with palmitic acid [3] and a non-significant increase in apoB when carbohydrates were replaced with palmitic acid but not

when replaced with stearic acid [4]. TAG concentrations were comparable between diets, which may suggest that the number of VLDL particles was unchanged. It is therefore of interest to examine if stearic acid induces a shift towards smaller and denser LDL particles. Furthermore, two of the four studies found decreased apoA1 concentrations [17, 19]. It is uncertain if this is associated with less (pre-β) HDL particles, since one HDL particle can contain up to four A1 apolipoproteins [53]. As apoA1 is involved in ATP-binding cassette transporter (ABC) A1-mediated cholesterol efflux from peripheral cells to pre-β-HDL particles, it is of interest to examine if these decreased apoA1 concentrations result in impaired reverse cholesterol transport. Only a few studies examined effects on hematological markers. Platelet volume decreased when minimally 5 en% palmitic acid was exchanged for stearic acid [22]. Total platelet count was not affected, which suggests smaller platelets that are considered to be less active than larger ones [54]. In addition, FVIIc activity decreased when 14 en% palmitic acid was exchanged for stearic acid [17] but not when 5 en% was exchanged [22]. Furthermore, the first study used shea butter, while the latter used hydrogenated canola oil. It has been suggested that the effects of shea butter may be due to its non-glyceride components instead of its stearic acid content [22]. Hematological markers related to fibrinolysis were not affected [17, 22]. Remarkably, only one of the longer-term studies included in this review has addressed the effects of palmitic and stearic acids on inflammation [20], and only two studies examined fasting glucose and insulin concentrations [12, 20]. In these studies, no differences were observed, but more research is needed to confirm these results.

Postprandial effects

Postprandial TAG responses are highly dynamic and depend on many factors. For example gender, age, and obesity are known to affect postprandial lipemia [55]. Indeed, the studies that included obese subjects observed higher postprandial TAG responses in obese compared with healthy-weight subjects [31, 38]. In addition, one study observed lower postprandial TAG concentrations in premenopausal women than in men [7]. Normally, TAG concentrations in the blood peak three to five hours after the meal and return to baseline within six to eight hours [56]. The studies included in this review differed in postprandial follow-up, ranging between four and eight hours. Since not only the peak value of TAG after a meal, but also the time to return to fasting TAG concentrations (the duration of lipemia) is positively related to CVD [55, 56], it may be important to follow-up for at least six hours to gain more insights in both peak values and duration of lipemia. In addition, during the day, people generally consume another meal after four to six hours. However, none of the studies included a so-called second meal challenge. Introducing a second fat-rich meal four to six hours after the first meal has been shown to induce the release of chylomicrons that contain fatty acids from the previous meal [57]. Therefore, meal effects may be affected by the composition of the previous meal. In addition, postprandial impairment of endothelium-

dependent vasodilation and oxidative stress are most marked after a second fat-containing meal [58]. Conflicting results have been reported on postprandial TAG responses of native and interesterified palmitic or stearic acid-rich fats. This discrepancy might be explained by the characteristics of the fats used, in particular the solid fat content at 37°C. In most studies, solid fat content increased if the proportion of palmitic acid or stearic acid at sn-2 increased. However, in one study solid fat content was lower for the fat blend high in palmitic acid at sn-2 and results of this study were opposite to those of other studies, e.g. higher TAG response after the fat with higher sn-2 palmitic acid content [28]. It has been suggested that solid fat content at body temperature rather than sn-2 palmitic or stearic acid content determines the postprandial TAG response [5]. It is hypothesized that a high solid fat content at 37°C, which is often due to tristearin (SSS) or tripalmitin (PPP) TAG species, impairs micelle formation [14] and reduces accessibility for pancreatic lipase [31], thereby decreasing the rate of absorption by the enterocyte. FVIIa responses seem to be related to postprandial lipemia, e.g. attenuated lipemia is associated with decreased FVIIa responses [33]. Although no changes in glucose and insulin responses were shown between fats differing in sn-2 palmitic or stearic acid content, results on postprandial release of gut hormone GIP were less clear. GIP induces insulin secretion and is released when fatty acids and/or carbohydrates enter the small intestine [59]. GIP has only been measured in studies investigating the sn-2 position of palmitic acid [28, 37], and results differed between these two studies. Palm oil increased GIP more than interesterified palm oil [37], while no difference was observed after the native and interesterified blend of palm stearin and palm kernel [28]. It is likely that this is due to the difference in physical characteristics of the control fats used; fats liquid at body temperature such as high oleic sunflower oil and palm oil increase GIP more than fats with solids at body temperature such as interesterified palm oil and lard [37]. Since both the native and interesterified blends of palm stearin and palm kernel were partly solid at body temperature, effects on GIP were possibly attenuated [28]. The only study that has measured the effects of positional distribution within the TAG molecules on postprandial inflammatory cytokines and E-selectin observed no effects of sn-2 palmitic acid content in a meal [7]. Substituting palmitic with stearic acid does not seem to affect postprandial responses of lipids and (apo)lipoproteins, although two studies observed a lower TAG response after lard compared with palm olein [40]. However, it is uncertain if this difference is due to the exchange between palmitic and stearic acid or due to differences in sn-2 content of palmitic acid and subsequently physical characteristics; lard has a higher solid fat content at 37°C. Postprandial effects on hematological markers, glucose-insulin homeostasis, and inflammation require further attention, but so far results do not indicate clear differences between palmitic and stearic acids.

Conclusions
Interesterification of palmitic acid- or stearic acid-rich fats does not seem to affect fasting serum lipids and (apo)lipoproteins. On the other hand, stearic acid decreases LDL- and HDL-cholesterol concentrations compared with palmitic acid. In addition, postprandial lipemia is attenuated if the changes in palmitic acid or stearic acid sn-2 contents increase solid fat content of the blend at body temperature. No evidence was found that solely substituting palmitic acid with stearic acid affected postprandial lipemia. However, there is need to further examine fasting and postprandial effects of (interesterification of) palmitic acid- and stearic acid-rich fats on the hemostatic system, inflammation, and glucose-insulin homeostasis as well as on emerging cardiometabolic risk markers such as cholesterol efflux capacity and lipoprotein particle size. In addition, it would be of interest for future studies to specifically examine populations that have a higher risk for CVD, such as elderly or people with obesity, and to examine sex differences as well.

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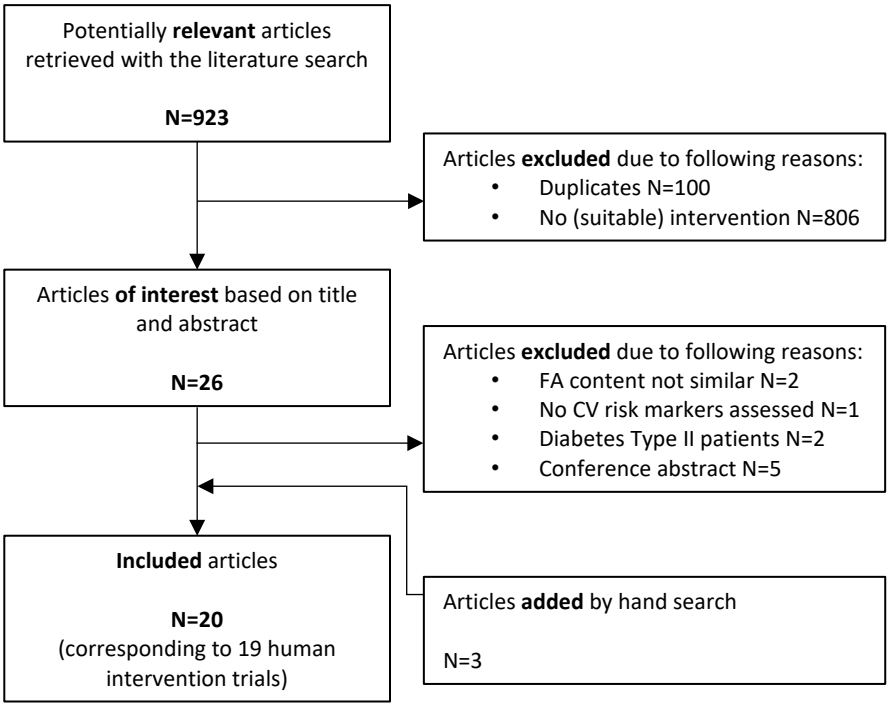
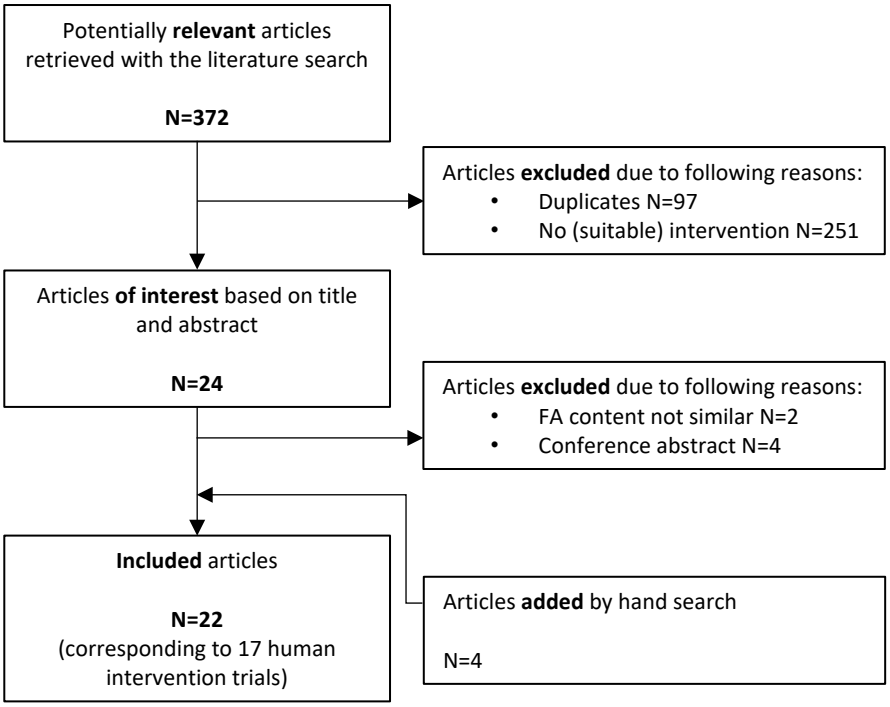
Supplemental data



Supplemental Figure 1. Flow chart of studies on the effects of interesterification of palmitic acid- or stearic acid-rich fats on cardiometabolic risk markers. Abbreviations: FA, fatty acids; CV, cardiovascular.



Supplemental Figure 2. Flow chart of studies on the effects of palmitic acid versus stearic acid on cardiometabolic risk markers. Abbreviations: FA, fatty acid.

Supplemental Table 1. Longer-term effects of substituting fats low in sn-2 palmitic acid (C16:0) contents with fats high in sn-2 C16:0 contents on fasting cardiometabolic risk. markers.

First author, Year of publication	Study population, Age, BMI	Duration intervention periods, Study design	Total fat (en%)	C16:0 (en%)	Source Low *sn*-2 High *sn*-2	C16:0 sn-2 in fat blends (%[a])	Solid fat at 37°C (%)	Lipids and lipoproteins	Hemato-logical markers	Other markers
Nestel, 1995 [10]	27 men (mildly hyperchol[$]) 49±8 y 26.3±2.5 kg/m^2	21 days Crossover (no WO)	31	6.7	Palm oil IE palm oil	8.7 24.7 wt%	NR	TAG = TC = LDL-C = HDL-C =		
Zock, 1995 [9]	23 men 37 women[$$] 29 (19-67) y 22.9 (18.1-30.9) kg/m^2	21 days Crossover (no WO)	40	11	Control and IE blend of palm oil blended with sunflower oil	6.4 66.9 wt%	0 0	TAG = TC =[*] LDL-C =[*] HDL-C =		
Meijer, 1997 [13]	30 men 30 women ±35.5 y ±23.8 kg/m^2	21 days Crossover[#] (no WO)	34	1 or 2[#]	Control and IE blend (mainly coconut and palm oils blended with soybean oil)	7.1 18.0 wt%	NR	TAG = NEFA = TC = LDL-C = HDL-C = Lp[a] =	FVIIa = Fibrinogen = PAI-1 antigen = tPA antigen = tPA activity = vWF =	Glucose = CRP =
Christophe, 2000 [8]	32 men 23-53 y 18.1-23.5 kg/m^2	28 days Parallel	NR ±131g	NR ±5g	IE butter Butter	NR	NR	TAG = TC = LDL-C= HDL-C= ApoB =		

								ApoA1 =	
Filippou, 2014 [11]	10 men 31 women ±29.1 y ±23.0 kg/m^2	42 days Crossover (no WO)	27	9	Palm olein IE palm olein	9.8 45.9 mol%	0 5.9	TAG = TC = LDL-C = HDL-C = ApoB = ApoA1 = Lp[a] =	Glucose = Insulin = C-peptide =
Ng, 2018 [12]	64 women 21 men 20-60y 21-30 kg/m^2	56 days Parallel	35	7	Palm olein CIE palm olein	11.1 32.4 wt%	NR	TAG = TC = LDL-C = HDL-C = ApoB = ApoA1 = Lp[a] =	Glucose = Insulin = C-peptide =

Markers are significantly lower (↓), higher (↑) or not significantly different (=) after intake of fats high in C16:0 sn-2 contents compared with fats low in C16:0 sn-2 contents. [a]=% of total fatty acids at sn-2. [$]=Subjects were mildly hypercholesterolemic (Average total cholesterol: 6.00 ± 0.78 mmol/L) [10]. [$$]=Pre- and postmenopausal women were included, however study was designed in such a way that menstrual cycle or use of oral contraceptives should not have influenced results [9]. [*]=In men, total and LDL cholesterol concentrations were slightly increased (0.10 mmol/L and 0.08 mmol/L respectively) on the diet with higher C16:0 sn-2 [9]. [#]=Subjects were divided into two parallel groups that were assigned to a diet with either 4 or 8 en% of the blends. Of the 60 subjects in total, 32 (16 men and 16 female) subjects followed the 4 en% diet (age ± 33 years, BMI: ± 24.1 kg/m2) and 28 (14 men and 14 female) subjects the 8 en% diet (age ± 38 years, BMI ± 23.4 kg/m2). The blends provided 1 and 2 en% palmitic acid in the 4 and 8 en% diet respectively, total amount of palmitic acid in the diets was not reported [13]. Abbreviations: apoB, apolipoprotein B; apoA1, apolipoprotein A1; CRP, CIE, chemically interesterified; C-reactive protein; en%, % of total energy; FVIIa, activated factor VII; HDL-C, high-density lipoprotein cholesterol; IE, interesterified; Lp[a], lipoprotein [a]; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; NR, not reported; PAI, plasminogen activator inhibitor; sn, stereospecific numbering; TAG, triacylglycerol; TC, total cholesterol; tPA, tissue plasminogen activator; vWF, von Willebrand Factor; WO, wash out period; wt, weight; y, year.

Supplemental Table 2. Longer-term effects of substituting fats low in sn-2 stearic acid (C18:0) contents with fats high in sn-2 C18:0 contents on fasting cardiometabolic risk markers.

First author, Year of publication	Study population, Age, BMI	Duration intervention periods, Study design	Total fat (en%)	C18:0 (en%)	Source Low sn-2 High sn-2	C18:0 sn-2 in fat blends (%[a])	Solid fat at 37°C (%)	Lipids and lipoproteins	Hemato-logical markers	Other markers
Grande, 1970 [15]	32 men 40-65 y NR	18 days Latin-square	34	10	Native or IE cocoa butter[$$] blended with safflower oil	NR	NR[$]	TAG = TC =		
Berry, 2007 [14]	16 men 26.8±8.0 y 23.7±3.7 kg/m^2	21 days Crossover	30g test fat#	7[#]	Native or IE shea butter blended with sunflower oil	3.1 22.8 mol%	22 41	TAG = TC = LDL-C = HDL-C =	FVIIa =	Glucose = Insulin =

Markers are significantly lower (↓), higher (↑) or not significantly different (=) after intake of fats high in C18:0 sn-2 contents compared with fats low in C18:0 sn-2 contents. [a]=% of total fatty acids at *sn*-2. [$]=Melting points of the blends were not measured. Authors reported that native cocoa butter is normally liquid at 37°C, while they calculated that IE cocoa butter should have 19% solid fat content at 40.5°C [15]. [$$]=the interesterified cocoa butter was a mix of palm oil, totally hydrogenated soybean oil, and olive oil which matched the fatty acid composition of native cocoa butter [15]. [#]=Total daily intake of total fat and stearic acid was not reported. Diets provided 30 grams of test fat and an additional 7 en% (15 grams) of C18:0 per day [14]. Abbreviations: en%, % of total energy; FVIIa, activated factor VII; HDL-C, high-density lipoprotein cholesterol; IE, interesterified; LDL-C, low-density lipoprotein cholesterol; NR, not reported; sn, stereospecific numbering; TAG, triacylglycerol; TC, total cholesterol; y, year.

Supplemental Table 3. Longer-term effects of substituting fats high in palmitic acid (C16:0) with fats high in stearic acid (C18:0) on fasting cardiometabolic risk markers.

First author, Year of publication	Study population, Age, BMI	Duration intervention period, Study design	Total fat (en%)	C16:0 C18:0 (en%)	Difference between diets C16:0 C18:0 (en%)	Main source C16:0 C18:0	Lipids and lipoproteins	Hematological markers	Other markers
Grande, 1970 [15]	32 men 40-65 y NR	18 days Latin-square	34	15 10	6 8	Palm oil Cocoa butter	TAG = TC ↓		
Bonanome, 1988 [16]	11 men 64±4.0 y 24±1.7 kg/m^2	21 days Cross-over (no WO)	40	18 17	15	Palm oil Hydro-genated soybean oil	TAG = TC ↓ VLDL-C = LDL-C ↓ HDL-C =		
Tholstrup, 1994 [17] + 1995 [25]	15 men 24.9 (22-30) y 23.2 (20.4-26.4) kg/m^2	21 days Cross-over	40	16$^{\$}$ 14	14	Palm oil Shea butter	TAG = TC ↓ VLDL-C = LDL-C ↓ HDL-C ↓ ApoB ↓ ApoA1 ↓ Lp[a] ↑	FVIIc ↓ PAI-1 activity = PAI-1 antigen = tPA activity = tPA antigen = EFA =	
Dougherty, 1995 [18]	10 men 37.4±6.6 y 25.2±2.5 kg/m^2	40 days Cross-over (no WO)	27-29	7	5 6	Palm oil Shea butter	TAG = TC ↓ LDL-C ↓ HDL-C =		

Schwab, 1996 [19] + 1997 [26]	12 women[$$] (premenopausal) 23.5±3.1 y 22.1±2.4 kg/m^2	28 days Cross-over	37	12 7	3 5	Palm oil, butter Cocoa butter	TAG = NEFA =[$$] TC ↓ VLDL-C = LDL-C = HDL-C ↓ ApoB = ApoA1 ↓		CETP activity ↓ Glucose =[$$] Insulin =[$$]
Nestel, 1998 [21]	15 subjects (mildly hyperchol men and women[#]) 51±7 y 26.2±3.9 kg/m^2	35 days Cross-over (no WO)	41-42	8[##]	±5	Palm olein Fully hydrogenated soybean oil	TAG = TC = LDL-C = HDL-C =		
Snook, 1999 [23]	16 women (premenopausal) 28±6 y NR	35 days 3x3 cross-over	40	13	10 11	Tripalmitin Tristearin	TAG = TC ↓ LDL-C ↓ HDL-C = ApoB = ApoA1 =		CETP activity = LCAT activity =
Kelly, 2001 [22]	13 men 35±12 y 26±3.3 kg/m^2	28 days Cross-over	27-28	8 7	6 5	Palm stearin and/or palm olein Hydrogenated canola	TAG = TC = LDL-C = HDL-C =	FVIIc = MPV ↓ Fibrinogen = Plasminogen = WBC = RBC = Hgb = PLT =	APTT = ATIII =

Kelly, 2002 [24]	9 men 39±10 y 25±2.5 kg/m^2	21 days Cross-over	28-29	7 4	1 2	Potato crisps, shortbread biscuits, muesli bars Milk chocolate	TAG = TC = LDL-C = HDL-C =	MPV = WBC = RBC = Hgb = PLT =	
Ng, 2018 [12]	64 women 21 men 20-60y 21-30 kg/m^2	56 days Parallel	35	7 8	5 7	IE Palm olein IE hydrogenated soybean oil	TAG ↓ TC = LDL-C = HDL-C = ApoB = ApoA1 = Lp[a] =		Glucose = Insulin = C-peptide =
Meng, 2019 [20]	20 postmenopausal women (mildly hypercholˆ) 64±7 y 26.4±3.4 kg/m^2	35 days Cross-over	30	14* 10	8 9	Palm oil Cocoa butter	TAG = TC ↓ VLDL-C = LDL-C ↓ HDL-C ↓ ApoB = ApoA1 = Lp[a] =	PT = PTT =	Glucose = Insulin = CRP = TNF-a = IL-6 = SAA-1 = sICAM-1 = sICAM-3 = sVCAM-1 = E-selectin = P-selectin = Thrombo-modulin =

Markers are significantly lower (↓), higher (↑) or not significantly different (=) after intake of fats high in C18:0 compared with fats high in C16:0. $Total dietary intake of C16:0 and C18:0 was not reported, values represent intakes from the blends. Blends provided 90% of total fat intake [17]. $$The measurements of glucose, insulin, and non-esterified fatty acids were performed in a sub study with 8 of the 12 participants. Glucose and insulin were assessed with an intravenous glucose tolerance test and due to technical reasons the results of only 6 subjects were available on both diets [26]. #Number of men and women that completed the study was not reported but 12 men and 8 women were screened. Not defined if women were pre- or postmenopausal. Subjects were mildly hypercholesterolemic (Average total cholesterol: 6.13 ± 0.80 mmol/L) [21]. ##Total dietary intake of C16:0 and C18:0 was not reported, values represent intakes from the blends. Blends provided 55% of total fat intake [21]. ^Mildly hypercholesterolemic based on LDL-cholesterol concentrations (Average LDL-cholesterol: 3.5 ± 0.7 mmol/L, total cholesterol: 5.6 ± 0.8 mmol/L) [20]. Abbreviations: apoB, apolipoprotein B; apoA1, apolipoprotein A1; APTT, activated partial thromboplastin time; ATIII, antithrombin III; CETP, cholesteryl ester transfer protein; CRP, C-reactive protein; EFA, euglobulin fibrinolytic activity; en%, % of total energy; FVIIc, Factor VII coagulant activity; HDL-C, high-density lipoprotein cholesterol; Hgb, hemoglobin; HOSO, high oleic acid sunflower oil; IE, interesterified; IL, interleukin; LCAT, lecithin-cholesterol acyltransferase; Lp[a], lipoprotein [a]; LDL-C, low-density lipoprotein cholesterol; MPV, mean platelet volume; NR, not reported; PAI, plasminogen activator inhibitor; PLT, platelet count; PT, prothrombin time; PTT, partial thormoplastin time; RBC, red blood cells; SAA, serum amyloid A; sICAM, soluble intercellular adhesion molecule; sn, stereospecific numbering; sVCAM, soluble vascular cell adhesion molecule; TAG, triacylglycerol; TC, total cholesterol; TNF, tumor necrosis factor; tPA, tissue plasminogen activator; VLDL, very low-density lipoprotein; WBC, white blood cells; WO, wash out period; wt, weight; y, year.

Supplemental Table 4. Postprandial effects of substituting fats low in palmitic acid (C16:0) sn-2 contents with fats high in C16:0 sn-2 contents on cardiometabolic risk markers.

First author, Year of publication	Population, Age, BMI, Follow-up	Total energy (kcal)	Total fat in grams (en%)	C16:0 content in grams (en%)	Source Low sn-2 High sn-2	C16:0 sn-2 in fat blends (%[a])	Solid fat at 37°C (%)	Lipids and lipopro-teins	Hemato-logical markers	Other markers
Zampelas, 1994 [35]	16 men 24.8±2.6 y 22.7±2.4 kg/m^2 6h	662	40 (54en%)	12 (16en%)	Palm olein IE blend of palm stearine with sunflower oil	5.9 72.7 wt%	NR	TAG = NEFA =		Glucose = Insulin = GIP =
Summers, 1998 [36]	2 men 6 women 30.5 (18-55) y 24 (19-30) kg/m^2 6h	932	60 (58en%)	18 (17en%)	NR	5.9 67.8 mol%	NR	TAG = NEFA =		Glucose = Insulin =
Yli-Jokipii, 2001 [32]	10 women (premenopausal) 26.9±2.56 y 18.5-25 kg/m^2 6h	NR	55g/m2 body surface area	17g/m2 body surface area	Palm oil IE palm oil	9 31 mol%	0 0	TAG ↓ NEFA = VLDL-C = CM-C =		Glucose = Insulin =
Yli-Jokipii, 2003 [27]	2 men 7 women (premenopausal) 24±3 y 21.5±2.5 kg/m^2 8h	NR	55g/m2 body surface area	17g/m2 body surface area	IE Lard Lard	52 69 mol%	11.0[$] 12.5	TAG =[#] NEFA = TC =		Glucose = Insulin =

Berry, 2007 [33]	20 men 28.8±10.3 y 23.2±2.6 kg/m^2 6h	853	50 (53en%)	14 (15en%)	Palm oil IE palm oil	7.2 37.2 mol%	3.6 15.2	TAG = TC = LDL-C = HDL-C =	FVIIa = WBC =	Glucose = Insulin =
Sanders, 2011 [7] Filippou, 2014 [37]	25 men 25 women (premenopausal) ±24.8 y ±23.5 kg/m^2 8h	846	50 (53en%)	20 (22en%)	Palm olein IE palm olein	9.2 39.1 mol%	0 4.7	TAG = NEFA = TC = ApoB48 =		Glucose = Insulin = C-peptide = GIP ↓ PYY= IL-6 = IL-8 = TNF-α = E-selectin =
Hall, 2014 [34]	11 men 50±7 y 27.6±3.1 kg/m^2 6h	1047	75 (64en%)	30 (26en%)	Palm olein IE palm olein	9.8 45.9 mol%	NR	TAG =^ NEFA = TC =		
Hall, 2017 [28]	12 men 20.5±1.1 y 22.4±2.8 kg/m^2 4h	832	52 56en%	26 28en%	PSt/PK IE PSt/PK	36.0 54.7 mol%	24^^ 21	TAG ↑		Glucose = Insulin = GIP = PYY =

Markers are significantly lower (↓), higher (↑) or not significantly different (=) after intake of fats high in C16:0 sn-2 contents compared with fats low in C16:0 sn-2 contents. [a]=% of total fatty acids at sn-2. [$]= 12.5% Lard and 11.0% IE lard was solid at 35°C, and 8.3% and 6.5% at 40°C respectively. No values reported for 37°C [27]. [#]=iAUC of VLDL-TAG was smaller after lard [27]. ^=TAG iAUC of 0 to 4 hours after IE palm olein was lower than after palm olein (p=0.024). Chylomicron TAG was lower at 4h after IE palm olein compared to palm olein (p=0.038) [34]. ^^= 24% PSt/PK and 21% IE PSt/PK was solid at 35°C, and 17 and 11% at 40°C respectively. No values for 37°C [28]. Abbreviations: apoB48, apolipoprotein B48; CM-C, chylomicron cholesterol; en%, % of total energy; FVIIa, activated factor VII; GIP, glucose-dependent insulinotropic polypeptide; HDL-C, high-density lipoprotein cholesterol; IE, interesterified; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; NR, not reported; PSt/PK, palm stearin blended with palm kernel; PYY, peptide YY; TAG, triacylglycerol; TC, total cholesterol; TNF, tumor necrosis factor; VLDL, very low-density lipoprotein; WBC, white blood cells; wt, weight; y, year.

Supplemental Table 5. Postprandial effects of substituting fats low in stearic acid (C18:0) sn-2 contents with fats high in C18:0 sn-2 contents on cardiometabolic risk markers.

First author, Year of publication	Population, Age, BMI, Follow-up	Total energy (kcal)	Total fat in grams (en%)	C18:0 content in grams (en%)	Source Low *sn*-2 High *sn*-2	C18:0 *sn*-2 in fat blends (%[a])	Solid at 37°C (%)	Lipids and lipoproteins	Hemato-logical markers	Other markers
Summers, 1999 [29]	14 women 49 (29-70) y 27.5 (20.6-52.8) kg/m^2 6h	932	60 (58en %)	18 (18en%)	NR	NR 83.3	NR	TAG = NEFA =		Glucose = Insulin =
Sanders, 2003 [30]	17 men 38.2±11.1 y 24.5±2.9 kg/m^2 6h	749	50 (60en %)	17 (20en%)	Cocoa butter IE cocoa butter	NR	NR[#]	TAG ↓ TC = LDL-C =	FVIIa ↓	
Berry, 2007 [14]	16 men 26.8±8.0 y 23.7±3.7 kg/m^2 8h	853	50 (53en %)	26 (28en%)	Native or IE shea butter blended with HOSO	3.1 22.8 mol%	22.2 41.2	TAG = NEFA = TC = LDL-C = HDL-C =	FVIIa = WBC =	Glucose = Insulin =
Robinson, 2009 [31]	10 non-obese men (55.8±7.0y, 26.6±2.5 kg/m^2) 11 obese men (59.3±6.0y, 32.9±4.3 kg/m^2), 6h	NR	86-102 (76en %) (1g/kg body mass)	25-30 (21en%)	Canola stearin (EIE, CIE, native) blended with HOSO	0.5 0.6 25.5 wt%	5.4 5.6 18.6	Non-obese: TAG = Obese: TAG ↓[$] Both: NEFA = TC = LDL-C =		Both: Glucose = Insulin =

HDL-C =

Markers are significantly lower (↓), higher (↑) or not significantly different (=) after intake of fats high in C18:0 *sn*-2 contents compared with fats low in C18:0 *sn*-2 contents. [a]=% of total fatty acids at *sn*-2. [#]=Melting points were 35 and 50°C for native and randomized cocoa butter, respectively [30]. [$]=The native blend (high C18:0 *sn*-2) had a lower TAG response compared to the chemically interesterified blend (low C18:0 *sn*-2) but not compared to the enzymatically interesterified blend [31]. Abbreviations: CIE, chemically interesterified; en%, % of total energy; EIE, enzymatically interesterified; FVIIa, activated factor VII; HDL-C, high-density lipoprotein cholesterol; HOSO, high oleic sunflower oil; IE, interesterified; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; NR, not reported; TAG, triacylglycerol; TC, total cholesterol; WBC, white blood cells; wt, weight; y, year.

Supplemental Table 6. Postprandial effects of substituting fats high in palmitic acid (C16:0) with fats high in stearic acid (C18:0) on cardiometabolic risk markers.

First author, year of publication	Population, Age, BMI, Postprandial follow-up	Total energy (kcal)	Total fat in grams (en%)	Content C16:0 C18:0 (g)	Content C16:0 C18:0 (en%)	Source C16:0 C18:0	Lipids and lipopro-teins	Hemato-logical markers	Other markers
Mennen, 1998 [42]	91 women (postmenopausal) 75.7±5.2 y 27.7±4.1 kg/m^2 6-7 hours	948-889	55.7-49.3 (53-50en%)	22 19	21 19	NR	TAG =	FVIIa =	
Jensen, 1999 [38]	15 women (premenopausal) 8 normal-weight (27±2 y, 19.2-23.7 kg/m^2) 7 overweight (29±3 y, 28.8-47.5 kg/m^2) 8 hours	406kcal/m^2 body surface area	29g/m^2 (65en%)	12g/m^2 5g/m^2	27 10	Palm oil Lard	Both: TAG =		Both: Insulin = Leptin =
Sanders, 2000 [39]	11 men 5 women (premenopausal) 25.5 (18-32) y 23.2 (20.1-27.8) kg/m^2 7 hours	1242	90 (65en%)	37 36	27 26	Palm oil Hydro-genated and IE HOSO	TAG =	FVIIa = FVIIc =	

Tholstrup, 2001 [41] + 2003 [44] + 2004 [43]	16 men 23.4±2.4 y 23±2 kg/m^2 8 hours	1672#	75# (50.6 en%##)	32# 34#	17 18	IE blend of tripalmitin or tristearin with HOSO	TAG = NEFA = VLDL-C = LDL-C = HDL-C = ApoB = ApoA1 = Lp[a] =	FVIIa = FVIIc = PAI-1 antigen = tPA activity =	CETP activity = LPL activity =	
Teng, 2011 [40]	10 men 21.9±0.7 y 21.0±1.6 kg/m^2 4 hours	754	50 (60en%)	17 9	21 10	Palm olein Lard	TAG ↓ NEFA =		Glucose = Insulin = Leptin =	IL-6 = TNF-α = IL-1ß =
Sanders, 2011 [7] Filippou, 2014 [37]	25 men 25 women (premenopausal) ±24.8y, ±23.5kg/m^2 8 hours	846	50 (53en%)	20 9	22 9	Palm olein Lard	TAG ↓ NEFA ↓ TC = ApoB48 =		Glucose = Insulin = C-peptide = GIP ↓ PYY=	IL-6 = IL-8 = TNF-α = E-selectin =

Markers are significantly lower (↓), higher (↑) or not significantly different (=) after intake of fats high in C18:0 compared with fats high in C16:0. #=per 75kg body weight. Range of fat intake was 65-85 grams [41]. ##=50.6 en% was reported. However, our calculations indicate 40.4 en% [41]. Abbreviations: apoB, apolipoprotein B; apoA1, apolipoprotein A1; CETP, cholesteryl ester transfer protein; en%, % of total energy; FVIIa, activated factor VII; FVIIc, Factor VII coagulant activity; GIP, glucose-dependent insulinotropic polypeptide; HDL-C, high-density lipoprotein cholesterol; HOSO, high oleic sunflower oil; IE, interesterified; IL, interleukin; Lp[a], lipoprotein [a]; LPL, lipoprotein lipase; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; NR, not reported; PAI, plasminogen activator inhibitor; PYY, peptide YY; TAG, triacylglycerol; TC, total cholesterol; TNF, tumour necrosis factor; tPA, tissue plasminogen activator; VLDL-C, very-low density lipoprotein cholesterol; wt, weight; y, year.

CHAPTER 3

Dietary palmitic acid and stearic acid do not differently affect ABCA1-mediated cholesterol efflux capacity in healthy men and postmenopausal women: a randomized controlled trial

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Submitted

Abstract

Background: The saturated fatty acid stearic acid (C18:0) lowers HDL cholesterol compared with palmitic acid (C16:0). However, the ability of HDL particles to promote cholesterol efflux from macrophages (cholesterol efflux capacity; CEC) may better predict coronary heart disease (CHD) risk than HDL cholesterol concentrations.

Objective: We examined effects of exchanging dietary palmitic acid for stearic acid on ATP-binding cassette transporter A1 (ABCA1)-mediated CEC, and other conventional and emerging cardiometabolic risk makers.

Design: In a double-blind, randomized, crossover study with two 4-week isocaloric intervention periods, 34 healthy men and postmenopausal women (61.5±5.7 years, BMI: 25.4±2.5 kg/m^2) followed diets rich in palmitic acids or stearic acids. Difference in intakes was 6% of daily energy. ABCA1-mediated CEC was measured from pCPT-cAMP treated J774 macrophages to apolipoprotein (apo)B-depleted serum.

Results: Compared with the palmitic-acid diet, the stearic-acid diet lowered serum LDL cholesterol (-0.14 mmol/L; p=0.010), HDL cholesterol (-0.09 mmol/L; p=<0.001), and apoA1 (-0.05 g/L; p<0.001). ABCA1-mediated CEC did not differ between diets (p=0.280). Cholesteryl ester transfer protein (CETP) mass was higher on stearic acid (0.11 mg/L; p=0.003), but CETP activity was comparable. ApoB100 did not differ, but triacylglycerol concentrations tended to be higher on stearic acid (p=0.100). Glucose concentrations were comparable. Effects on insulin and C-peptide were sex-dependent. In women, the stearic-acid diet increased insulin concentrations (1.57 μU/mL; p=0.002), while in men, C-peptide concentrations were lower (-0.15 ng/mL; p=0.037). Interleukin 6 (0.15 pg/mL; p=0.039) and tumor necrosis factor alpha (0.18 pg/mL; p=0.005), but not high-sensitivity C-reactive protein, were higher on stearic acid. Soluble intracellular adhesion molecule (9 ng/mL; p=0.033), but not soluble vascular cell adhesion molecule and endothelial-selectin concentrations decreased after stearic-acid consumption.

Conclusions: As expected, stearic-acid intake lowered LDL cholesterol, HDL cholesterol, and apoA1. Insulin sensitivity in women and low-grade inflammation might be unfavorably affected by stearic-acid intake. However, palmitic-acid and stearic-acid intakes did not differently affect ABCA1-mediated CEC.

Introduction

Palmitic acid (C16:0) and stearic acid (C18:0) are the most abundant saturated fatty acids (SFA) in many Western diets [1]. It is well known that these two SFA differently affect lipid metabolism. Compared with palmitic acid, stearic acid lowers concentrations of total cholesterol (TC) and LDL cholesterol as well as those of HDL cholesterol [2-4]. Related to coronary heart disease (CHD), the decrease in LDL cholesterol is beneficial as each 1 mmol/L reduction in LDL cholesterol is associated with a 21% reduction in major vascular events [5]. The effects of the decrease in HDL cholesterol are more controversial. For long, low HDL cholesterol concentrations were thought to be causally related to an increased CHD risk [6]. However, recent drug intervention studies have shown that increasing the level of HDL cholesterol by inhibiting cholesteryl ester transfer protein (CETP) does not necessarily decrease cardiovascular events such as CHD mortality [7]. These findings have contributed to the hypothesis that not the concentration of HDL cholesterol, but the functionality of HDL particles is important. One important function of HDL as related to CHD risk may be its ability to promote cholesterol efflux from lipid-loaded macrophages in the arterial wall. Indeed, a recent meta-analysis showed an inverse relationship between HDL-mediated cholesterol efflux capacity (CEC) and cardiovascular events [8]. Interestingly, this relation was independent of conventional cardiovascular risk markers such as HDL cholesterol [8, 9]. Whether palmitic acid and stearic acid differentially affect CEC is not known. The main aim of this study, therefore, was to investigate the effects of palmitic acid and stearic acid on HDL-mediated CEC. In addition, we examined effects on other conventional and emerging cardiometabolic risk markers including lipids, (apo)lipoproteins, CETP mass and activity, markers of glucose homeostasis, endothelial function, and low-grade systemic inflammation.

Methods

Participants

Healthy men and women were recruited between April 2016 and February 2017 from Maastricht and surrounding areas via advertisements in local newspapers and Maastricht University Medical Centre+ (MUMC+). The last participant completed the study on July 1, 2017. Persons who were interested received detailed information about the study and were invited for a screening visit if they met the following criteria: aged between 45 and 70 years, postmenopausal (women), BMI between 18 and 30 kg/m^2 with a stable body weight during the last three months (<3 kg change), no cardiovascular disease or medical condition that might interfere with the study outcomes, consumption of <10 (women) or <14 (men) alcoholic beverages per week, <10 hours exercise per week, no consumption of plant sterol- or stanol-enriched products during the last three months, non-smoker or ≤7 cigarettes per week, no night shifts, and no blood donation or participation in other biomedical trials within 12 weeks before the start of (and during) the study, and not working at Unilever or MUMC+. During the screening visit, participants first had to give their written informed consent. Then,

weight and height were determined, and a fasted blood sample was obtained via venipuncture. Lastly, participants had to complete a general and medical questionnaire. Participants were included if they were healthy based on the medical questionnaire, had fasted serum TC concentrations < 8.0 mmol/L and TAG concentrations < 4.5 mmol/L, and plasma glycosylated hemoglobin A1c (HbA1c) concentrations < 48 mmol/mol (or 6.5%).

Study design

This double blind, randomized, crossover trial consisted of two 4-week intervention periods in which participants consumed experimental foods containing either palmitic acid (C16:0)- or stearic acid (C18:0)-rich fat blends. Before randomization, participants were stratified for gender. The randomization list, which was generated by an independent biostatistician, was based on a computer-generated scheme in which a diet order code was assigned to a subject number. Participants were allocated to one of the two diet orders by the dietician. If couples participated, they were both allocated to the same diet order. Intervention periods were separated by a washout period of at least 4 weeks during which participants consumed their habitual diets. The Medical Ethical Committee of the MUMC+ had approved the protocol and the study was registered at ClinicalTrials.gov with identifier NCT02835651.

Intervention diets

Before the start of the study, daily energy requirement of each subject was estimated with the Harris-Benedict equation [10] multiplied by an estimated average physical activity level of 1.4. Based on these estimates, participants were assigned to a diet providing 1950, 2250, 2550 or 2850 kilocalories per day. The daily amounts of experimental foods that participants consumed during the intervention periods were based on the calculated daily energy requirements. Intervention diets consisted of 35% daily energy (en%) as fat, of which 16 en% was provided by either the palmitic acid- or stearic acid-rich fat blends incorporated into the experimental foods. Intake of the experimental foods was aimed to result in a 6% difference in energy intake between palmitic acid and stearic acid. The dietician provided guidelines to ensure that - except for the intakes of stearic acid and palmitic acid - total energy and nutrient intakes were comparable between the diets. For the remaining 19 en% as fat, participants had to consume a predefined amount of 'free-choice' fat-containing products that had to be chosen from a list with products that were assigned points. Each point equaled 1 gram of fat and participants were instructed to stay within a predefined range of points based on their daily energy requirement. Participants were required to record daily their choices and corresponding points into a food diary. Diaries were checked weekly by the dietician, who also advised the participants in case of any problems or questions. In addition, participants noted daily how many of the experimental foods they had consumed, and if they had used any medications, had consumed alcohol, had any signs of illness or had deviated from the study protocol. At the end of both intervention periods, participants completed a food

frequency questionnaire (FFQ) in which they had to fill in total food intake from the previous four intervention weeks. These FFQs were checked by the dietician and used to estimate total energy and nutrient intakes using the Dutch food composition table (NEVO).

Experimental fat blends and foods

Experimental foods rich in palmitic acid or stearic acid were prepared with blends of natural fats provided by Unilever R&D (Vlaardingen, Netherlands). For the palmitic acid-rich blend, a mix of 90% palm oil mid-fraction and 10% high-oleic sunflower oil was used. For the stearic acid-rich blend, a mix of 92% allanblackia oil and 8% sunflower oil was used. Proportions of total saturated, monounsaturated, and polyunsaturated fatty acids were comparable between the fat blends (Supplemental Table 1). Slip melting points (SMP) for the palmitic acid- and stearic acid-rich blend were respectively 33.9°C and 40.5°C, and the solid fat contents at 37°C were 1 and 8%. The experimental foods included buns, cookies, and lemon curd, and were made by the bakery department of the Hotelschool Hasselt in Belgium. The products were color-coded based on the corresponding blend in order to blind participants and investigators. Only the dietician knew which color belonged to which blend. Products were prepared in four batches after which buns and curds were stored at -20°C and cookies at controlled room temperature. Participants received new products at each visit, except at the last visit of each intervention period.

Measurements

Participants were asked not to change their level of physical exercise and alcohol consumption during the study. During each 4-week intervention period, participants visited the research unit at the university at days 0, 14, 25, and 28. They were asked to travel to the university by public transport or by car to minimalize physical activity, and to refrain from alcohol and consume their last meal before 20:00h the day prior to these visits. At each visit, the same participant visited the research unit at approximately the same time. Participants were weighed and fasted blood samples were collected via venipuncture. Blood pressure was measured four times using an Omron M7 (Omron Healthcare Europe BV, Hoofddorp, The Netherlands), of which the last three measurements were averaged. If body weight changed >1.5 kg from initial body weight (at day 0) during the first week or >2 kg from initial body weight during the following weeks, energy intake and the corresponding amounts of experimental food products were adjusted accordingly to prevent further changes in weight.

Blood was sampled at days 0, 25, and 28 in serum separator vacutainer tubes (Becton, Dickinson and company, NJ, USA) for analyses of CEC, CETP mass and activity, TC, HDL cholesterol, apolipoprotein A1 (apoA1), apoB100, apoCII, apoCIII, triacylglycerol (TAG), apoB48, non-esterified fatty acids (NEFA), insulin, C-peptide, and high-sensitivity C-reactive protein (hsCRP). After sampling, serum tubes were allowed to clot for at least 30 minutes at room temperature and subsequently centrifuged at 1300×g for 15 minutes at 20°C. Blood

for glucose analysis was sampled in NaF-plasma vacutainer tubes (Becton, Dickinson, and Company) and blood for interleukin (IL)-6, tumor necrosis factor (TNF)-α, soluble intracellular adhesion molecule (sICAM)-1, soluble vascular cell adhesion molecule (sVCAM)-1, and endothelial (E)-selectin in K_2EDTA- plasma vacutainer tubes (Becton, Dickinson, and Company). After sampling, the tubes for NaF- and EDTA-plasma preparation were directly put on ice and subsequently centrifuged at 1300×g for 15 minutes at 4°C. Aliquots of serum and plasma samples were snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Biochemical analyses

Samples from one subject were analyzed within the same analytical run. All parameters were measured at days 0, 25, and 28 from both intervention periods.

Concentrations of TC and HDL cholesterol after precipitation of apoB-containing lipoproteins using tungstophosphoric acid hydrate (Merck, Kenilworth, NJ, USA) and magnesium chloride (enzymatically; Roche Diagnostics Systems, Mannheim, Germany), TAG corrected for free glycerol (enzymatically; Sigma-Aldrich, St. Louis, MO, USA), apoA1 and apoB100 (immunoturbidimetrically; Horiba ABX, Montpellier, France), apoCII and apoCIII (immunoturbidimetrically; Randox Laboratories, Crumlin, UK), apoB48 (ELISA; Shibayagi Co., Shibukawa, Japan), and NEFA (enzymatically; Wako Chemicals GmbH, Neuss, Germany) were measured in serum samples. LDL cholesterol concentrations [11], and the ratios of TC:HDL cholesterol and apoB100:apoA1 were calculated.

Ex vivo CEC, CETP mass, and CETP activity were also measured in serum samples. The ability of HDL particles to remove cholesterol from macrophages, the CEC, was quantified ex vivo as previously described [12]. Briefly, J774 macrophages were seeded in a 96-wells plate (50.000 cells/well) and incubated overnight at 37°C and 5% CO_2 to load them with 24.42 µg/mL BODIPY-cholesterol (INstruchemie, Delfzijl, Netherlands). Acyl-CoA Acyltransferase (ACAT)-inhibitor (1 µL/mL; Sigma-Aldrich) was present during all assay steps to inhibit esterification of cholesterol. After loading, ATP-binding cassette transporter A1 (ABCA1) was upregulated overnight with 4 µL/mL pCPT-cAMP (Sigma-Aldrich). This was followed by addition of 2.8 µL/mL apoB-depleted serum from a subject diluted in PBS. A negative control without serum was included on each plate. After 4 hours incubation, cholesterol efflux was quantified. To normalize CEC values, a pooled sample from serum of healthy people was included on each plate. The CEC value of this pool was set to 100% and subject values were expressed relative to the pool (% pool). CETP mass was determined using an ELISA (Cloud-Clone Corp., Houston, TX, USA) and CETP activity fluorometrically using synthetic lipid donor and acceptor particles (Roar Biomedical, NYC, NY, USA).

Serum insulin and C-peptide (radioimmunoassay (RIA); Linco Research, St. Charles, MO, USA), and plasma glucose (enzymatically; HORIBA ABX) concentrations were also measured and the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated [13].

Concentrations of hsCRP (immunoturbidimetrically; HORIBA ABX) were measured in serum, and IL-6, TNF-α, sICAM-1, sVCAM-1, and E-selectin (multiplex immunoassay; Meso Scale Diagnostics, Rockville, MD, USA) in plasma.

Statistical analyses

This study was powered on changes in CEC, which was the primary outcome parameter of the study. In an earlier study (unpublished data) we found that the within-subject variability of ex vivo CEC was 18%. The statistical power to detect a true difference of at least 9% between diets using a two-side alpha of 0.05 was over 80% with 32 participants. This number of participants also provided a statistical power of >90% to demonstrate expected differences in fasting TC, LDL-C, and HDL-C concentrations [14]. To compensate for an estimated dropout rate of 20%, 41 participants were included. For all parameters, values of days 25 and 28 were averaged before statistical analyses. Results were considered statistically significant when the p-value was ≤ 0.05.

Effects of palmitic versus stearic acid on CEC, lipids, (apo)lipoproteins, CETP mass and activity, glucose, insulin, C-peptide, HOMA-IR, sICAM-1, and sVCAM-1 were compared using a linear mixed model with participants as random factor, period and diet as fixed factors, and baseline values of both the corresponding period as well as the average baseline of both periods as covariates. The sex*diet and BMI*diet interaction terms were also included in the model as fixed factors, but removed if the term did not reach statistical significance. For BMI, participants were categorized based on a BMI <25 kg/m^2 or ≥25 kg/m^2. Differences between intervention periods are reported as estimated differences with 95% confidence interval (CI). Dietary intakes between both intervention periods were compared using a linear mixed model with participants as random factor, and period and diet as fixed factors. Effects on inflammatory markers and E-selectin were reported as medians with ranges (25th-75th percentiles), because residuals were not normally distributed as indicated by the Shapiro-Wilk test. For these variables, changes (average day 25 and 28 minus baseline concentrations) were calculated and the difference in changes was tested using the Wilcoxon signed-rank test. All statistical analyses were performed using IBM SPSS Statistics for Mac, version 24.0 (IBM Corp. Armonk, NY, USA). Blinding was maintained until all analyses were performed.

Results

Participants and compliance

Forty-one participants were included and randomized after screening. During the first week of the first intervention period, six participants withdrew because they experienced the intervention as too burdensome and one subject due to diagnosis with Parkinson's disease. Thus, 34 participants (20 men and 14 women) completed the study and were included in the statistical analyses (Supplemental Figure 1). Their baseline characteristics are shown in Table 1. Of these 34 participants, 10 men and 7 women started with the palmitic-acid diet followed by the palmitic-acid diet, and the other 10 men and 7 women consumed the two diets in reverse order. According to the protocol and after blind review, two participants were excluded for the per-protocol analyses. One man because he lost too much weight (-3.9 kg, equaling 6.5% of total body weight) and one woman because she was prescribed antibiotic treatment. Results were however not different between the intention-to-treat analyses (n=34) and per-protocol (n=32) analyses and therefore only results of the intention-to treat-analyses are reported.

Based on the recorded daily intake of the experimental food products, average compliance was 99.4% on the palmitic-acid diet and 99.8% on the stearic-acid diet. Compared with the palmitic-acid diet, intake of palmitic acid was 6.0 en% lower on the stearic-acid diet ($p<0.001$) and intake of stearic acid 6.5 en% higher ($p<0.001$; Table 2). Oleic-acid intake was slightly higher during the stearic-acid diet period (+0.4 en%; $p=0.019$) and fiber intake was -1.3 grams lower ($p=0.041$). Changes in body weight ($p=0.072$), systolic and diastolic blood pressure ($p=0.750$ and $p=0.520$ respectively), and heart rate ($p=0.254$) were not different between the two diets (Supplemental Table 2).

Table 1. Baseline characteristics of participants who completed the study[1].

	All participants (n=34)	Men (n=20)	Women (n=14)
Age (y)	61.5 ± 5.7	61.3 ± 5.7	61.9 ± 6.6
BMI (kg/m^2)	25.4 ± 2.5	25.5 ± 1.8	25.3 ± 3.4
Total cholesterol (mmol/L)	5.60 ± 1.01	5.53 ± 1.09	5.71 ± 0.96
HDL cholesterol (mmol/L)	1.68 ± 0.39	1.54 ± 0.34	1.87 ± 0.40
Total:HDL cholesterol	3.5 ± 1.1	3.8 ± 1.1	3.2 ± 1.0
Triacylglycerol (mmol/L)	1.12 ± 0.52	1.27 ± 0.62	0.92 ± 0.28
HbA1c (mmol/mol)	37.3 ± 3.9	37.4 ± 4.5	37.1 ± 3.3

[1]Values are means ± SD. HbA1c, glycosylated hemoglobin A1c.

Table 2. Composition of the diets during the intervention periods[1].

	C16:0-rich diet	C18:0-rich diet	P-value[2]
Energy (kcal)	2448 ± 310	2414 ± 313	0.282
Carbohydrates	46.3 ± 3.8	45.9 ± 3.9	0.464
Protein	15.3 ± 1.3	15.1 ± 1.5	0.388
Fat	36.4 ± 3.3	36.9 ± 3.6	0.250
SFA	15.5 ± 1.7	15.7 ± 1.8	0.257
C16:0	10.2 ± 0.9	4.1 ± 0.5	<0.001
C18:0	2.2 ± 0.3	8.7 ± 0.9	<0.001
Cis-MUFA	14.0 ± 1.5	14.3 ± 1.8	0.167
C18:1	11.3 ± 1.5	11.6 ± 1.8	0.019
Cis-PUFA	5.3 ± 1.0	5.2 ± 1.0	0.332
C18:2 n-6	4.3 ± 0.8	4.2 ± 0.8	0.289
C18:3 n-3	0.64 ± 0.24	0.68 ± 0.23	0.122
Alcohol	2.0 ± 2.1	2.0 ± 2.1	0.420
Cholesterol (mg)	371 ± 49	368 ± 51	0.633
Fiber (g)	29.3 ± 4.4	28.1 ± 3.7	0.041

[1]Values are means ± SD and expressed in % of energy, unless otherwise noted. Values were obtained from Food Frequency Questionnaires.

[2]Differences between the two intervention periods were analyzed using linear mixed models.

HDL-metabolism

Ex vivo ABCA1-mediated CEC of serum HDL particles did not differ between diets (p=0.280; **Table 3**). The concentration of serum CETP mass increased with 0.11 mg/L on the stearic-acid diet compared with palmitic acid (p=0.003), but ex vivo CETP activity was comparable between the two diets (p=0.482).

Lipids and lipoprotein metabolism

Compared with the palmitic-acid diet, the stearic-acid diet lowered serum concentrations of TC (-0.20 mmol/L; p=<0.001), of LDL cholesterol (-0.14 mmol/L; p=0.010), and of HDL cholesterol (-0.09 mmol/L; p=<0.001; **Table 4**). The ratio of TC to HDL cholesterol increased by 0.13 on the stearic-acid diet (p=0.002). Serum TAG concentrations were not significantly different between the diets, but tended to be higher on stearic-acid intake (0.08 mmol/L; p=0.100). Serum concentrations of apoA1 decreased by 0.05 g/L on the stearic-acid diet (p<0.001), while those of serum apoB100 were not significantly different (p=0.133). Consequently, the ratio of apoB100 to apoA1 was increased by 0.02 on the stearic-acid diet compared with the palmitic-acid diet (p=0.024). Concentrations of serum apoCII (p=0.899), apoCIII (p=0.843), apoB48 (p=0.732), and NEFA (p=0.423) were comparable.

Table 3. Fasted concentrations of markers related to HDL metabolism on diets rich in stearic acid versus palmitic acid.

		Means ± SD[1]		Difference with 95% CI[2]	P-value
	Diet	Baseline	End		
CEC (% pool)	C16:0	96.6 ± 6.4	94.9 ± 7.2	1.0 (-0.8, 2.8)	0.280
	C18:0	98.9 ± 7.0	95.9 ± 6.8		
CETP activity	C16:0	60.4 ± 20.6	60.5 ± 22.4	1.1 (-1.9, 4.0)	0.482
(pmoles transferred)	C18:0	62.4 ± 22.5	61.3 ± 18.2		
CETP mass (mg/L)	C16:0	2.07 ± 0.48	1.97 ± 0.45	0.11 (0.04, 0.18)	0.003
	C18:0	1.97 ± 0.43	2.10 ± 0.45		

[1]Baseline and End concentrations are expressed as unadjusted means ± SD (n=34).
[2]Differences between the palmitic acid- and stearic acid-rich diets, expressed as least squared means with 95% confidence interval (95% CI), were estimated using linear mixed models.
Abbreviations: CEC, cholesterol efflux capacity; CETP, cholesteryl ester transfer protein.

Glucose metabolism

Plasma glucose concentrations were comparable between the two diets (p=0.242; **Table 5**). However, effects of the diets on insulin, C-peptide, and the HOMA-IR were different between men and women. In women, serum insulin concentrations were 1.57 μU/mL higher on the diet rich in stearic acid (p=0.002), while in men no differences were observed (p=0.440). For concentrations of serum C-peptide, no significant differences were observed in women although concentrations tended to be higher on the stearic-acid diet (+0.14 ng/mL; p=0.068). In men, however, C-peptide concentrations were -0.15 ng/mL lower on the stearic-acid diet (p=0.037). In women, the HOMA-IR was 0.42 units higher on the stearic-acid diet (p=0.002), while it did not differ in men (p=0.398).

Low-grade inflammation and endothelial function

Plasma concentrations of IL-6 and TNF-α were increased on the stearic-acid diet (+0.15 pg/mL; p=0.039 and +0.18 pg/mL; p=0.005 respectively; **Table 6**). No differences between the diets were observed for concentrations of serum hsCRP. Concentrations of plasma E-selectin (p=0.675) and sVCAM-1 (p=0.087) were not significantly different between diets, while sICAM-1 was 9 ng/mL lower on the stearic-acid diet than on the palmitic-acid diet (p=0.033).

Table 4. Fasted concentrations of lipids and (apo)lipoproteins on diets rich in stearic acid versus palmitic acid.

		Means ± SD[1]		Difference with 95% CI[2]	P-value
	Diet	Baseline	End		
Total cholesterol (mmol/L)	C16:0	5.57 ± 1.17	5.58 ± 1.29	-0.20 (-0.31, 0.10)	<0.001
	C18:0	5.61 ± 1.12	5.39 ± 1.22		
LDL cholesterol (mmol/L)	C16:0	3.51 ± 1.12	3.58 ± 1.22	-0.14 (-0.24, -0.03)	0.010
	C18:0	3.58 ± 1.12	3.44 ± 1.14		
HDL cholesterol (mmol/L)	C16:0	1.48 ± 0.33	1.48 ± 0.29	-0.09 (-0.12, -0.06)	<0.001
	C18:0	1.48 ± 0.34	1.39 ± 0.27		
Total:HDL cholesterol	C16:0	3.98 ± 1.28	3.93 ± 1.20	0.13 (0.05, 0.22)	0.002
	C18:0	3.98 ± 1.21	4.06 ± 1.29		
Triacylglycerol (mmol/L)	C16:0	1.29 ± 0.63	1.16 ± 0.57	0.08 (-0.02, 0.18)	0.100
	C18:0	1.19 ± 0.53	1.24 ± 0.62		
ApoB100 (g/L)	C16:0	1.14 ± 0.30	1.13 ± 0.30	-0.02 (-0.04, 0.01)	0.133
	C18:0	1.15 ± 0.28	1.12 ± 0.29		
ApoA1 (g/L)	C16:0	1.52 ± 0.18	1.50 ± 0.15	-0.05 (-0.07, -0.03)	<0.001
	C18:0	1.52 ± 0.16	1.45 ± 0.15		
ApoB100:ApoA1	C16:0	0.77 ± 0.25	0.76 ± 0.22	0.02 (0.00, 0.03)	0.024
	C18:0	0.77 ± 0.21	0.78 ± 0.23		
ApoCII (mg/dL)	C16:0	5.00 ± 2.09	4.70 ± 2.00	0.01 (-0.20, 0.23)	0.899
	C18:0	5.00 ± 2.03	4.71 ± 2.21		
ApoCIII (mg/dL)	C16:0	10.0 ± 2.99	9.35 ± 2.68	0.03 (-0.30, 0.36)	0.843
	C18:0	9.74 ± 2.30	9.34 ± 2.78		
ApoB48 (mg/L)	C16:0	5.09 ± 2.65	5.13 ± 2.97	0.06 (-0.31, 0.44)	0.732
	C18:0	4.80 ± 2.60	5.13 ± 2.56		
NEFA (μmol/L)	C16:0	364 ± 123	344 ± 135	-16 (-55, 23)	0.423
	C18:0	356 ± 144	328 ± 150		

[1]Baseline and End concentrations are expressed as unadjusted means ± SD (n=34). [2]Differences between the palmitic acid- and stearic acid-rich diets, expressed as least squared means with 95% confidence interval (95% CI), were estimated using linear mixed models. Abbreviations: Apo, apolipoprotein; NEFA, non-esterified fatty acids.

Table 5. Fasted concentrations of glucose and insulin parameters on diets rich in stearic acid versus palmitic acid.

	Sex (m/w)		Means ± SD[1]		Difference with 95% CI[2]	P-value
		Diet	Baseline	End		
Glucose (mmol/L)	All	C16:0	5.57 ± 0.38	5.45 ± 0.35	0.04 (-0.03, 0.11)	0.242
		C18:0	5.56 ± 0.38	5.49 ± 0.36		
Insulin (μU/mL)[3]	M	C16:0	14.10 ± 4.80	13.45 ± 4.56	-0.47 (-1.66, 0.73)	0.440
		C18:0	13.25 ± 5.38	13.11 ± 4.82		
	W	C16:0	11.56 ± 3.65	11.17 ± 3.41	1.57 (0.61, 2.53)	0.002
		C18:0	11.51 ± 3.46	12.75 ± 4.51		
C-peptide (ng/mL)[3]	M	C16:0	2.17 ± 0.62	2.17 ± 0.64	-0.15 (-0.28, -0.01)	0.037
		C18:0	2.12 ± 0.60	2.06 ± 0.56		
	W	C16:0	1.92 ± 0.66	1.96 ± 0.59	0.14 (-0.01, 0.28)	0.069
		C18:0	1.95 ± 0.61	2.09 ± 0.83		
HOMA-IR[3]	M	C16:0	3.52 ± 1.25	3.31 ± 1.24	-0.13 (-0.44, 0.18)	0.398
		C18:0	3.35 ± 1.44	3.22 ± 1.25		
	W	C16:0	2.83 ± 0.96	2.69 ± 0.88	0.42 (0.17, 0.68)	0.002
		C18:0	2.80 ± 1.01	3.12 ± 1.20		

[1]Baseline and End concentrations are expressed as unadjusted means ± SD (n=34).

[2]Differences between the palmitic acid- and stearic acid-rich diets, expressed as least squared means with 95% confidence interval (95% CI), were estimated using linear mixed models.

[3]A statistically significant diet*sex interaction was found for insulin (p=0.011), C-peptide (p=0.006), and HOMA-IR (p=0.009). Therefore, results are reported for men (n=20) and women (n=14) separately.

Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance.

Table 6. Fasted concentrations of inflammation and endothelial function markers on diets rich in stearic acid versus palmitic acid.

		Means [1,2]		Difference with 95% CI[2]	P-value
	Diet	Baseline	End		
hsCRP (mg/L)[1]	C16:0	0.97 (0.48 – 2.09)	0.91 (0.46 – 2.83)	-0.01 (-0.54 – 0.83)	0.879
	C18:0	0.96 (0.43 – 2.01)	0.82 (0.57 – 1.68)		
IL-6 (pg/mL)[1]	C16:0	0.64 (0.46 – 0.82)	0.54 (0.40 – 0.82)	0.15 (-0.01 – 0.39)	0.039
	C18:0	0.49 (0.42 – 0.71)	0.60 (0.47 – 0.78)		
TNF-α (pg/mL)[1]	C16:0	2.21 (1.93 – 2.66)	2.06 (1.82 – 2.50)	0.18 (-0.01 – 0.48)	0.005
	C18:0	2.02 (1.82 – 2.74)	2.11 (1.82 – 2.65)		
E-selectin (ng/mL)[1]	C16:0	7.23 (4.52 – 9.02)	6.12 (4.49 – 9.35)	0.64 (-1.78 – 2.13)	0.675
	C18:0	6.49 (4.71 – 8.71)	6.77 (4.39 – 9.65)		
sVCAM-1 (ng/mL)[2]	C16:0	619 ± 102	615 ± 114	9 (-1, 19)	0.087
	C18:0	621 ± 120	624 ± 116		
sICAM-1 (ng/mL)[2]	C16:0	366 ± 73	362 ± 80	-9 (-17, -1)	0.033
	C18:0	367 ± 90	352 ± 72		

[1]Baseline and End concentrations are expressed as median with ranges (25-75th percentiles; n=34) because hsCRP, IL-6, TNF-α, and E-selectin were not normally distributed. Differences in changes between the diets were tested with a Wilcoxon signed-rank test.

[2]Baseline and End concentrations are expressed as unadjusted means ± SD (n=34). Differences between the palmitic acid- and stearic acid-rich diets, expressed as least squared means with 95% confidence interval (95% CI), were estimated using linear mixed models.

Abbreviations: E-selectin, endothelial-selectin; hsCRP, high-sensitivity C-reactive protein; sICAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule.

Discussion

In this double-blind, randomized, crossover study with healthy men and women, we confirmed that stearic acid lowers serum LDL cholesterol and HDL cholesterol compared with palmitic acid [2]. However, *ex vivo* ABCA1-mediated cholesterol efflux from cholesterol-loaded J774 macrophages to apoB-depleted serum was not affected by exchanging dietary palmitic acid for stearic acid. We deliberately focused on ABCA1-mediated CEC as a measure of cholesterol efflux, as this is in humans the predominant pathway of cholesterol efflux from cholesterol-enriched macrophages in the arterial wall [9]. Also, epidemiological studies suggest that ABCA1-mediated CEC is inversely related to CHD risk [15, 16].

The number of human studies describing the effects of dietary SFA on CEC is limited. In two studies [17, 18], the effects of diets rich in SFA, MUFA or n-6 PUFA on CEC were compared. SFA was provided by either palm oil [17], cheese or butter [18]. The exchange of fatty acids was about 8 [17] and 6 [18] en% and diets were fed for 4 to 5 weeks, which is comparable to our study. In both studies, effects were sex-dependent. In healthy men, Montoya et al. [17] observed no differences between the diets on whole-serum CEC from rat Fu5AH hepatoma cells. In women, however, effects of the SFA- and MUFA-diets on CEC were comparable, but values on these two diets were significantly lower than those on the n-6 PUFA diet. A fish oil-enriched n-3 PUFA diet was also included, which increased CEC compared with SFA in both men and women. Brassard et al. [18] found that in abdominally obese men, total CEC from J774 macrophages to apoB-depleted serum was increased on the Butter-diet compared with the Cheese- and PUFA-diets, but not compared with the MUFA-diet. In women, the Butter- and Cheese-diet similarly affected CEC, but CEC was higher on the MUFA- and PUFA-diets. Taken together, these two studies do not provide unequivocal evidence that CEC is different between diets rich in SFAs, MUFAs or plant-based PUFAs. However, effects may be sex-dependent, while the food matrix may be important as well.

Our results now suggest that the long-chain SFAs palmitic acid and stearic acid do not differently affect ABCA1-mediated CEC. Unlike in the two other studies with SFA-rich diets [17, 18], effects were not sex-dependent. Possibly, the statistical power of the present study was too low to detect such a sex difference. Another explanation may be that all women in this study were postmenopausal, while in the other two studies also premenopausal women were included. In a small study, CEC capacity was higher in premenopausal women than in men [19], but these findings could not be confirmed in a larger study [20]. Thus, it is unclear if menopause affects CEC. In addition, if dietary effects on CEC differ between pre- and postmenopausal women needs to be studied. Our finding that lower HDL cholesterol and apoA1 concentrations on the stearic-acid diet did not attenuate ABCA1-mediated CEC, supports previous studies that ABCA1-mediated CEC does not solely depend on concentrations of HDL cholesterol and apoA1 [15]. Whether this suggests that the concentration of pre-β-HDL particles - which are the preferred cholesterol acceptor in

ABCA1-mediated CEC - was not different between palmitic-acid and stearic-acid diets warrants further study.

Besides the expected decrease in HDL-cholesterol and apoA1 concentrations [2, 14], the diet rich in stearic acid also lowered concentrations of serum TC and LDL cholesterol compared with palmitic acid, which is also in line with earlier studies [2, 14]. Serum concentrations of apoB100 were comparable, while those of TAG tended to be higher on the stearic-acid diet. Based on a recent meta-analysis [2], a decrease in apoB100 was expected on the diet rich in stearic acid. Possibly, the amount of palmitic acid exchanged for stearic acid in the present study was not large enough to detect a significant effect on apoB100. Indeed, a study with 14 en% exchange observed lower apoB100 concentrations on stearic-acid intake [21], while three other studies with an exchange between 5 and 10 en% found no significant differences [22-24]. Effects on CETP activity - but not CETP mass - have been measured in two earlier studies with pre-menopausal women. CETP activity on the stearic-acid diet was lower in one study [23] and tended to be lower in the other study [24]. In the present study, however, no differences were observed in CETP activity although serum CETP mass was higher on the stearic-acid diet. As in other studies [25], we did find a positive association between CETP mass and CETP activity (data not shown). Apolipoproteins CII and CIII, which determine lipoprotein lipase (LPL) activity, were not different between the diets. Effects of palmitic acid and stearic acid on LPL activity have only been compared in one acute postprandial study, in which also no differences were observed [26].

Despite comparable effects on fasting glucose concentrations between the two diets, the stearic acid-rich diet increased fasting insulin concentrations in women, but no differences were observed in men. C-peptide concentrations also tended to be higher in women on the stearic acid-rich diet, but were significantly lower in men. These results may suggest that stearic acid increased insulin synthesis in women to compensate for decreased insulin sensitivity. Indeed, the HOMA-IR was also higher on the stearic-acid diet in women while in men no differences between the two diets were observed. Similar to our findings, three other studies in which palmitic acid was exchanged for stearic acid [27-29] also observed no differences in effects on fasting glucose. However, concentrations of insulin [27-29], C-peptide [27], and HOMA-IR [27, 29] did also not differ in these studies. One other study reported increased fasting glucose and decreased insulin concentrations when palmitic acid was exchanged for stearic acid [30]. However, the stearic-acid fat also provided slightly more SFA, less MUFA, and more PUFA [30], which makes it difficult to compare those results with the results of the present study. Taken together, although not consistent, there is some evidence that high stearic-acid intake unfavorably affects fasting glucose homeostasis in postmenopausal women.

Stearic-acid intake increased plasma concentrations of IL-6 and TNF-α, while in other studies no effects were reported [29, 31]. Remarkably, concentrations of sICAM-1 on the stearic acid-rich diet were decreased, while sVCAM-1 concentrations tended to increase.

These results seem to be contradictory as changes in pro-inflammatory cytokines are expected to be in the same direction as those of sICAM-1, and sVCAM-1 [32, 33]. Meng et al., however, did not demonstrate differences in concentrations of these endothelial markers [29]. Concentrations of E-selectin and hsCRP were not different, which is in line with two other studies [29, 31]. Thus, like for glucose homeostasis, effects of stearic-acid intake compared with palmitic-acid intake on low-grade inflammation and endothelial function are not uniform and more research in these areas is certainly warranted.

This study has several strengths, including its high dietary compliance as evidenced by the expected decreases in serum LDL-C and HDL-C concentrations. Because of the used fat blends and experimental foods that were specifically designed for this study, we are confident that effects are attributable to the exchange of palmitic acid for stearic acid. In addition, a broad spectrum of cardiometabolic risk markers was included. However, it should be noted that no gold standard exists to measure CEC. Assays to determine ex vivo CEC differ in many aspects, such as the used cholesterol donor and acceptor, incubation time, and type of labeled cholesterol. This makes it difficult to make comparisons between studies. However, studies that reported an inverse relationship between ABCA1-mediated CEC and cardiovascular disease risk [15, 16, 34] used a comparable method as ours. Finally, our study was not specifically powered to detect sex-differences and these results should therefore be interpreted with caution.

In conclusion, consumption of a stearic acid-rich diet for four weeks lowered LDL cholesterol, HDL cholesterol, and apoA1, and increased CETP mass as compared with a palmitic acid-rich diet. Insulin sensitivity in women and some inflammatory cytokines might be unfavorably affected by stearic-acid intake. However, palmitic-acid and stearic-acid intakes did not differently affect ABCA1-mediated CEC. How these findings translate to CHD risk needs further investigation.

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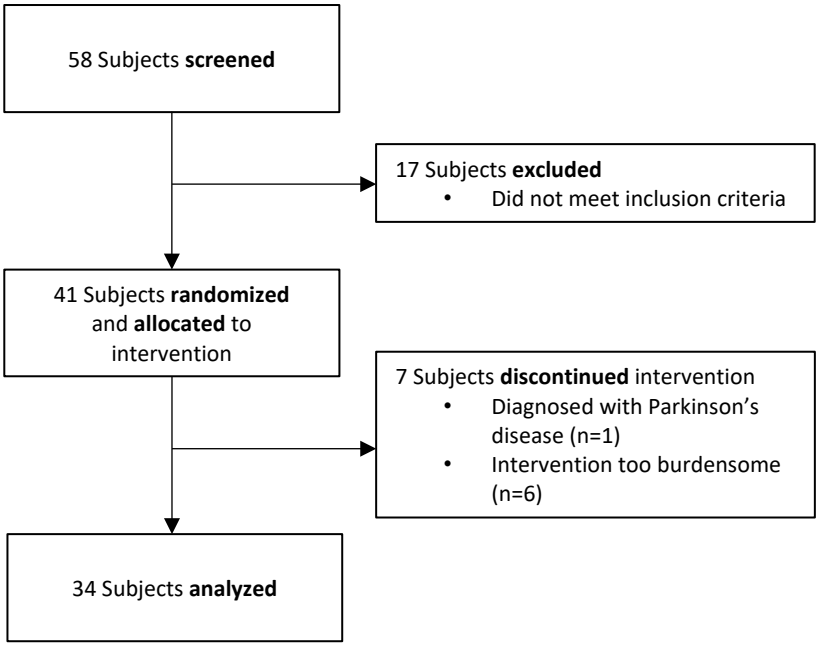
Supplemental data



Supplemental Figure 1. Flow chart of subject inclusion

Supplemental Table 1. Fatty acid composition (w/w) of the experimental blends[1].

	C16:0-rich blend	C18:0-rich blend
Fat blend	0.9 POM/0.1 HOSO	0.92 AB/0.08 SO
SFA (g/100g fat)	50.2	50.1
C16:0	43.5	3.2
C18:0	4.6	46.1
Cis-MUFA (g/100g fat)	38.8	39.4
C18:1	38.6	39.2
Cis-PUFA (g/100g fat)	7.0	6.9
C18:2 n-6	6.7	6.1
C18:3 n-3	0.2	0.6
SMP (°C)	33.9	40.5
Solid fat 37°C (%)	1	8

[1]Amounts of fatty acids are expressed as gram fatty acids (corrected for glycerol) per 100 grams fat. Abbreviations: AB, allanblackia oil; HOSO, high-oleic sunflower oil; POM, palm oil mid-fraction; SMP, slip melting point; SO, sunflower oil.

Supplemental Table 2. Body weight, blood pressure and heart rate on diets rich in stearic acid versus palmitic acid.

		Means ± SD[1]		Difference with 95% CI[2]	P-value
	Diet	Baseline	End		
Weight (kg)	C16:0	76.2 ± 11	76.1 ± 10.9	-0.2 (-0.4; 0.0)	0.072
	C18:0	76.6 ± 10.7	76.1 ± 10.7		
SBP (mmHg)	C16:0	121 ± 17	121 ± 19	0.4 (-1.9; 2.6)	0.750
	C18:0	120 ± 17	121 ± 18		
DBP (mmHg)	C16:0	78 ± 10	78 ± 10	0.4 (-0.9; 1.7)	0.520
	C18:0	78 ± 9	79 ± 9		
HR (mmHg)	C16:0	65 ± 11	64 ± 10	0.7 (-0.5; 2.0)	0.254
	C18:0	63 ± 9	64 ± 10		

[1]Baseline and End concentrations are expressed as unadjusted means ± SD (n=34).
[2]Differences between the palmitic acid- and stearic acid-rich diets, expressed as least squared means with 95% confidence interval (95% CI), were estimated using linear mixed models.
Abbreviations: DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure.

CHAPTER 4

Effects of two consecutive mixed meals high in palmitic acid or stearic acid on 8-hour postprandial lipemia and glycemia in healthy men and postmenopausal women

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To be submitted

Abstract

Background: The two most commonly consumed saturated fatty acids - palmitic acid and stearic acid - have different effects on fasting serum lipoprotein concentrations. However, effects of these two saturated fatty acids on postprandial lipemia and glycemia are less clear. In addition, effects of a second, consecutive meal on postprandial metabolism may differ from those of a single meal. Therefore, we studied effects of two consecutive mixed meals high in palmitic acid- or stearic acid-rich fat blends on postprandial lipemia and glycemia.

Design: This postprandial study was part of a randomized, crossover study in which healthy-weight or overweight men and women followed 4-week diets rich in palmitic acids or stearic acids. At the end of each period, participants consumed two consecutive meals each containing ±50 grams of the fat blend that was also consumed the preceding 4 weeks. Nineteen men and 13 women completed the postprandial test. Blood was sampled for 8-hours and the second meal was provided 4 hours after the first meal.

Results: Postprandial concentrations of triacylglycerol (diet-effect: -0.18 mmol/L; p=0.001) and apolipoprotein B48 (diet-effect: -0.68 mg/L; p=0.002) were lower after stearic-acid intake than after palmitic-acid intake. Consequently, the total ($iAUC_{0\text{-}8h}$) and first meal ($iAUC_{0\text{-}4h}$) responses were lower after stearic-acid intake for triacylglycerol and apolipoprotein B48 ($p \leq 0.01$). Second meal responses ($iAUC_{4\text{-}8h}$) were not different. Postprandial changes in non-esterified fatty acids (NEFA) and C-peptide differed over time ($p<0.001$ and p=0.020 for diet*time respectively) and the same tendency was observed for glucose (diet*time, p=0.074). Insulin changes were not different. The $dAUC_{0\text{-}8h}$, $dAUC_{0\text{-}4h}$, and $dAUC_{4\text{-}8h}$ for NEFA were larger after stearic acid ($p \leq 0.05$). No differences were observed in the iAUCs of C-peptide, glucose, and insulin. However, second meal responses for glucose and insulin ($iAUC_{4\text{-}8h)}$ tended to be lower after stearic-acid intake ($p<0.10$).

Conclusion: Consumption of the stearic acid-rich fat lowered postprandial lipemia as compared with palmitic acid, possibly due to its higher solid fat content at 37°C. Differences in parameters related to postprandial glycemia were smaller, but were more evident after the second meal, i.e. the stearic acid-rich fat resulted in a faster increase in C-peptide and more pronounced decrease in NEFA. Translation of these findings into health effects on the long-term needs further study.

Introduction

As we spend most of the day in a postprandial state, understanding relations between diet-induced postprandial physiological changes and cardiometabolic health is important. Indeed, it has been shown that elevated and prolonged postprandial lipemia and glycemia are associated with an increased risk to develop cardiovascular disease (CVD) [1, 2]. However, for dietary fat intake, recommendations are mainly based on effects on fasting serum LDL-cholesterol (LDL-C) concentrations, an established CVD-risk factor [3]. It is for example well-known that replacing saturated fatty acids with unsaturated fatty acids has a beneficial effect on LDL-C [4]. Saturated fat, however, is an umbrella term for different saturated fatty acids that exert different metabolic effects. Of these, palmitic acid (C16:0) and stearic acid (C18:0) are the most commonly consumed saturated fatty acids in the Western diet. It is well established that palmitic acid increases fasting serum LDL-C concentrations compared with stearic acid. However, effects of these saturated fatty acids on postprandial metabolism are less clear. Attenuated postprandial lipemia after acute intake of stearic acid compared with palmitic acid has been observed in two studies [5, 6], but not in other studies [7-11]. One hypothesis is that stearic acid-rich fats delay fat digestion and absorption, because of the presence of more fat solids at body temperature due to its higher melting range [12]. So far, no differences between palmitic-acid and stearic-acid intake have been found in postprandial responses of glucose [6, 13] and insulin [5, 8, 13].

In daily-life, people generally consume multiple meals a day and lipids ingested during the first meal will also appear in the circulation when a second meal is consumed, even if this second meal is low in fat [14, 15]. Previous studies examining acute effects of palmitic-acid versus stearic-acid intakes provided a low-fat lunch after 3 to 4 hours [6-9]. However, as most meals during the day provide fats, it is of interest to examine if postprandial responses of consecutive meals high in fat differ from those after a single meal. Therefore, we have examined the effects of two consecutive mixed meals high in palmitic acid- or stearic acid-rich fat blends on postprandial lipemia and glycemia during an 8-hour period. Postprandial tests were performed after subjects had consumed 4-week diets rich in the corresponding fatty acid.

Methods

This postprandial intervention study was part of a double-blind, randomized, crossover study that consisted of two 4-week intervention periods during which healthy-weight or overweight men and women received products enriched with either palmitic acids (C16:0) or stearic acids (C18:0). Intervention periods were separated by a wash-out period of at least 4 weeks. At the end of each intervention period, an 8-hour postprandial test was performed for which participants consumed a mixed meal high in either palmitic acid- or stearic acid-rich fat blends. Participants received a second meal 4 hours after the first meal to induce a second-meal effect. Effects of the 4-week diets on fasting cardiometabolic risk markers have been described previously (**Chapter 3**).

Participants

Briefly, healthy men and women were recruited from Maastricht and surrounding areas and met the following criteria: aged between 45 and 70 years, postmenopausal (women), BMI between 18 and 30 kg/m^2 with a stable body weight during the last three months (<3 kg change), no cardiovascular disease or medical condition that might interfere with the study. Participants were included if they were healthy, which was based on a medical questionnaire, had fasted serum total cholesterol (TC) concentrations < 8.0 mmol/L and triacylglycerol (TAG) concentrations < 4.5 mmol/L, and plasma HbA1c concentrations < 48 mmol/mol (or 6.5%). After screening, 41 participants were included. All participants gave their written informed consent before entering the study. The Medical Ethical Committee of the MUMC+ had approved the protocol. The study was registered at ClinicalTrials.gov with identifier NCT02835651.

Study design and meals

For the postprandial test at the end of each intervention period, participants were asked to refrain from strenuous exercise 48 hours before this test day. After measuring weight, blood pressure, and obtaining a fasted blood sample via venepuncture, an intravenous cannula was placed in an antecubital vein and another fasting blood sample was collected (T0). Participants then received a mixed meal provided as a shake, which they were asked to consume within 5 minutes. This meal contained 46.6 grams of the fat blend that participants also received during the preceding 4-week intervention period. The composition of the meal was similar for all participants and each meal consisted of 50 grams of fat, 5 grams of protein, and 54 grams of carbohydrates (**Table 1**). This amount of fat was chosen, because it represents a realistic fat load in a Western dinner and causes a clear increase in serum TAG concentrations [16]. 4 Hours after the first meal, participants consumed a second meal with the same composition as the first one. Postprandial blood samples were taken at 15 (T15), 30 (T30), 45 (T45), 60 (T60), 90 (T90), 120 (T120), 180 (T180), 240 (T240), 300 (T300), 360 (T360), 420 (T420), and 480 (T480) minutes after shake consumption. Immediately after

T240 (approximately around lunch time), the second meal was consumed. During the entire test day, participants were not allowed to drink anything – except for water – or to eat. Participants were asked to keep the amount of water consumption comparable between both test days.

Table 1. Nutrient composition of the standardized test shakes provided for breakfast and lunch.

	Palmitic acid-rich shake	Stearic acid-rich shake
Energy (kcal)	697.4	697.4
Carbohydrates (en%)	30.8	30.8
Protein (en%)	4.8	4.8
Fat (en%)	64.5 (50g)	64.5 (50g)
SFA (en%)	33.0	33.0
C16:0 (en%)	28.3 (22g)	3.1 (2.5g)
C18:0 (en%)	3.2 (2.4g)	29.2 (22.6g)
MUFA (en%)	26.0	26.4
C18:1 (en%)	25.7	26.1
PUFA (en%)	4.7	4.7
C18:3 n-3 (en%)	0.1	0.4
Cholesterol (mg)	120	120
Fiber (g)	1.28	1.28

SFA: saturated fatty acids, MUFA: cis-monounsaturated fatty acids, PUFA: cis-polyunsaturated fatty acids.

Experimental fat blends

Both blends of natural fats were provided by Unilever R&D (Vlaardingen, Netherlands). For the palmitic acid-rich blend, a mix of 90% palm oil mid-fraction (POM) and 10% high oleic sunflower oil (HOSO) was used. For the stearic acid-rich blend, a mix of 92% allanblackia oil (AB) and 8% sunflower oil (SO) was used. Fat blends were comparable in saturated, monounsaturated and polyunsaturated fatty acid content (**Supplemental Table 1**). Slip melting points for the palmitic acid- and stearic acid-rich blend were respectively 33.9°C and 40.5°C, and the solid fat contents at 37°C were 1 and 8%.

Blood collection and biochemical analyses

Blood was sampled in serum separator vacutainer tubes (Becton, Dickinson and company, NJ, USA) for analyses of triacylglycerol (TAG), apolipoprotein B48 (apoB48), non-esterified fatty acids (NEFA), insulin, and C-peptide. After sampling, serum tubes were allowed to clot for at least 30 minutes at room temperature and subsequently centrifuged at 1300×g for 15 minutes at 20°C. Blood for glucose analysis was sampled in NaF-plasma vacutainer tubes (Becton, Dickinson and company) and directly put on ice after sampling with subsequent centrifugation at 1300×g for 15 minutes at 4°C. Aliquots of serum and plasma samples were snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Serum concentrations of TAG corrected for free glycerol (GPO Trinder; Sigma-Aldrich, Missouri, USA) and apoB48 (ELISA; Shibayagi Co., Shibukawa Japan) were measured at all timepoints except for T15 and T45. Concentrations of serum NEFA (Wako Chemicals GmbH, Neuss, Germany), serum insulin and C-peptide (Linco Research, Missouri, USA), and plasma glucose (Horiba ABX) were measured at all time points. Samples from one subject were analyzed within the same analytical run.

Statistical analyses

Data are reported as least squared mean (LSM) with 95% confidence interval (CI) unless otherwise indicated. Postprandial time curves were analyzed using linear mixed models with participants as between subject variable, baseline values of the corresponding day (T0) as covariate, and period, diet, time, diet*time, and baseline as fixed factors. If the diet*time interaction term did not reach statistical significance, indicating that responses were similar at all time points, it was omitted from the model. In this model, statistical significance of the factor diet indicated that differences between the stearic acid and palmitic acid diets were similar at all time points. Differences are reported as least squared means (LSM) with 95% confidence interval (CI). Sex effects were also determined by addition of sex, diet*sex, time*sex, and diet*sex*time as fixed factors to the model. However, for none of the parameters sex effects were present and therefore omitted from the model. Incremental areas under the curve (iAUC) or decremental areas under the curve (dAUC) were calculated for all parameters using the trapezoidal rule as previously described [17]. We assessed the total postprandial response (0 to 8 hours; $i/dAUC_{0\text{-}8h}$), as well as the first meal response (0 to 4 hours; $i/dAUC_{0\text{-}4h}$) and the second meal response (4 to 8 hours; $i/dAUC_{4\text{-}8h}$). Peak increases or decreases were calculated by comparing maximal changes during the 8 hours postprandial follow-up to T0 ($max_{0\text{-}8h}$). Maximal changes after the first meal were calculated by comparing concentrations between T0 and T240 to the concentrations at T0 ($max_{0\text{-}4h}$) and maximal changes after the second meal were calculated by comparing concentrations between T240 and T480 to the concentrations at T240 ($max_{4\text{-}8h}$). i/dAUC differences and maximal increases were assessed using linear mixed models with subject as random factor, and period and diet as fixed factors, and reported as LSM with 95% CI. Sex effects were also tested for i/dAUCs by addition of sex and diet*sex to the model as fixed factors but the diet*sex interaction term was not significant for any of the parameters and thus omitted. Results were considered statistically significant if $p<0.05$. All analyses were performed using IBM SPSS Statistics for Mac, version 24.0 (IBM Corp. Armonk, NY, USA). Blinding was maintained until all analyses were performed.

Results

The participant flow throughout the study is shown in **Supplemental Figure 1**. Fifty-eight participants were assessed for eligibility of which 41 were included and randomly allocated to the intervention periods. Seven participants withdrew during the first week of the first intervention period as described previously (**Chapter 3**). Of the remaining 34 participants, one man and one woman did not complete one or both postprandial test day(s) due to nausea. In the end, 32 participants (19 men and 13 women) completed both postprandial test days and were included in the analyses. Of these, 10 men and 7 women started with the palmitic-acid diet, and 9 men and 6 women with the stearic-acid diet. Characteristics of the participants at screening are shown in **Supplemental Table 2**.

Postprandial lipemia

For postprandial TAG concentrations, there were no significant differences between the meals rich in palmitic acid or stearic acid at the various time points of the postprandial response (p=0.742 for diet*time interaction; **Figure 1**). However, a significant diet-effect was observed, i.e. postprandial TAG concentrations after stearic-acid intake were on average 0.18 mmol/L lower (p=0.001) over the total 8-h follow-up period. Consequently, the $iAUC_{0\text{-}8h}$ (p=0.002) and peak values ($TAG_{max0\text{-}8h}$; p=0.003) were lower after stearic-acid intake (**Supplemental Table 3**). During the 4 hours after the first meal, comparable results were observed as over the whole 8-hour follow-up period, i.e. the $iAUC_{0\text{-}4h}$ and $TAG_{max0\text{-}4h}$ were lower (p=0.007 for both) after the stearic-acid meal. The $iAUC_{4\text{-}8h}$ in the last 4 hours after the second meal was not statistically different between palmitic acid and stearic acid (p=0.127), but peak values tended to be lower after stearic-acid intake ($TAG_{max4\text{-}8h}$; p=0.079).

Differences in postprandial changes at the various timepoints for apoB48 were comparable (p=0.451 for diet*time interaction; **Figure 2**). Like for TAG, average apoB48 concentrations were overall lower after stearic-acid than after palmitic-acid intake (diet-effect: -0.68 mg/L; p=0.002), as also shown by the lower $iAUC_{0\text{-}8h}$ (p=0.008) and peak values ($ApoB48_{max0\text{-}8h}$; p=0.034; **Supplemental Table 4**). After the first meal, also a lower $iAUC_{0\text{-}4h}$ (p=0.010) and $ApoB48_{max0\text{-}4h}$ (p=0.048) were observed after intake of stearic acid. After the second meal, the $iAUC_{4\text{-}8h}$ (p=0.355) and $ApoB48_{max4\text{-}8h}$ (p=0.585) were not different between palmitic-acid and stearic-acid intakes.

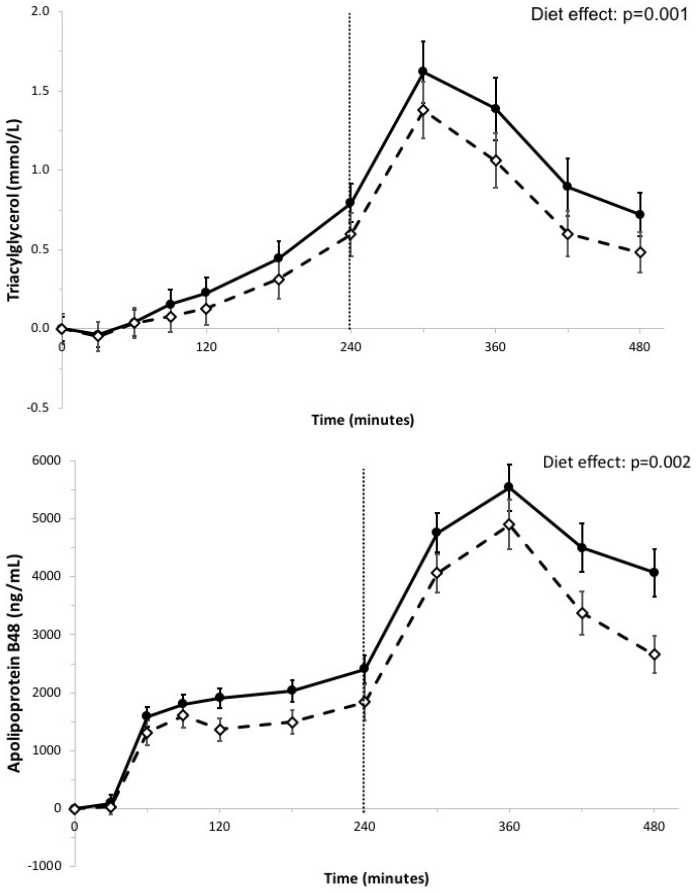

Figure 1 and 2. Postprandial changes in triacylglycerols (TAG; mmol/L) and apolipoprotein B48 (apoB48; ng/mL) over time after meals rich in palmitic acid (●) or stearic acid (◊). TAG concentrations were measured at baseline, and 30, 60, 90, 120, 180, 240, 300, 360, and 480 minutes after meal intake. After 240 minutes, a second meal was consumed that was similar to the first meal. N=32. A significant overall diet effect was observed (p=0.001).

Postprandial glycemia

For postprandial glucose concentrations, postprandial responses after palmitic-acid and stearic-acid intakes at the various time points tended to differ (p=0.074 for diet*time interaction; **figure 3**). However, no diet-effects were observed (p=0.503). The $iAUC_{0\text{-}8h}$ (p=0.375) and peak values ($Glucose_{max0\text{-}8h}$; p=0.876) were comparable between palmitic-acid and stearic-acid intakes (**Supplemental Table 5**). After the first meal, the $iAUC_{0\text{-}4h}$ (p=0.362) was also not different, but the $Glucose_{max0\text{-}4h}$ tended to be higher after stearic-acid intake (p=0.059). In contrast to the 4 hours after the first meal, the $iAUC_{4\text{-}8h}$ (p=0.095) and the $Glucose_{max4\text{-}8h}$ (p=0.064) after the second meal tended to be lower after stearic-acid intake.

Differences in postprandial changes at the various timepoints in insulin concentrations were comparable between the palmitic-acid and stearic-acid meals (p=0.248 for diet*time interaction; **Figure 4**), and also no diet-effects were observed (p=0.636). The $iAUC_{0\text{-}8h}$ (p=0.404) and $Insulin_{max0\text{-}8h}$ (p=0.483) did not differ between palmitic-acid and stearic-acid intakes, and similar results were observed during the 4 hours after the first meal (**Supplemental Table 6**). After the second meal, the insulin $iAUC_{4\text{-}8h}$ tended to be lower after intake of stearic acid (p=0.064), while $insulin_{max4\text{-}8h}$ was not significantly different (p=0.115).

Postprandial changes in C-peptide concentrations at the various timepoints differed between palmitic acid and stearic acid (p=0.020 for diet*time interaction; **Figure 5**). Compared with palmitic acid, stearic-acid intake resulted in significantly higher C-peptide concentrations 30 and 300 minutes postprandially (+1.01 ng/mL; p=0.011 and +1.16 ng/mL; p=0.004 respectively), but in lower concentrations at 420 minutes (-0.96 ng/mL; p=0.015). No differences between palmitic-acid and stearic-acid intakes were found in iAUCs and peak values over the total 8-h follow up, or in those over the 4h follow up after the first and second meals (**Supplemental Table 7**).

The postprandial time curves of NEFAs were different between the palmitic-acid and stearic-acid meals (p<0.001 for diet*time interaction; **Figure 6**). NEFA concentrations were lower after the stearic-acid meals at T300 (-177 μmol/L; p<0.001), T360 (-181 μmol/L; p<0.001), and T420 (-111 μmol/L; p=0.001). The postprandial $dAUC_{0\text{-}8h}$ (p=0.005) and maximal decrease of NEFA ($NEFA_{max0\text{-}8h;}$ p=0.026) were larger after stearic-acid intake (**Supplemental Table 8**). Similarly, in the 4 hours after the first meals, the $dAUC_{0\text{-}4h}$ was larger after intake of stearic acid (p=0.025) and the maximal decreases tended to be larger ($NEFA_{max0\text{-}4h}$; p=0.054). After the second meal, the $dAUC_{4\text{-}8}$ was also larger after stearic-acid intake (p=0.054), but the maximal decreases did not differ ($NEFA_{max4\text{-}8h}$; p=0.499).

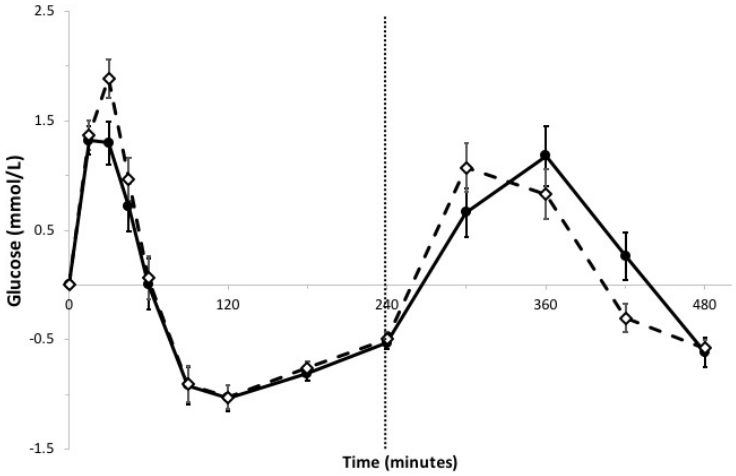

Figure 3. Postprandial changes in glucose (mmol/L) over time after meals rich in palmitic acid (•) or stearic acid (◊). Glucose concentrations were measured at baseline, and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes after meal intake. After 240 minutes, a second meal was consumed that was similar to the first meal. N=32.

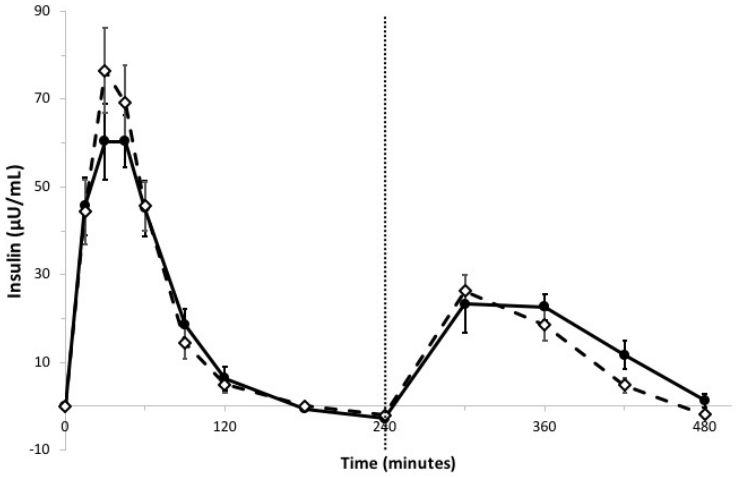

Figure 4. Postprandial changes in insulin (µU/mL) over time after meals rich in palmitic acid (•) or stearic acid (◊). Insulin concentrations were measured at baseline, and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes after meal intake. After 240 minutes, a second meal was consumed that was similar to the first meal. N=32.

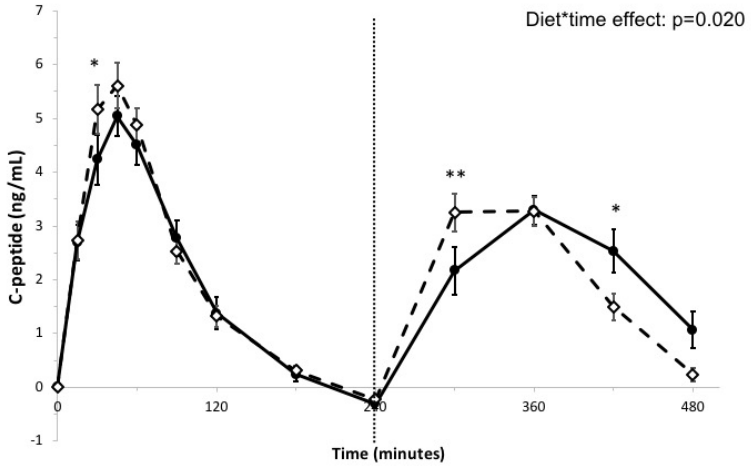

Figure 5. Postprandial changes in C-peptide (ng/mL) over time after meals rich in palmitic acid (•) or stearic acid (◊). C-peptide concentrations were measured at baseline, and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes after meal intake. After 240 minutes, a second meal was consumed that was similar to the first meal. N=32. A significant diet*time interaction was observed (p=0.020).

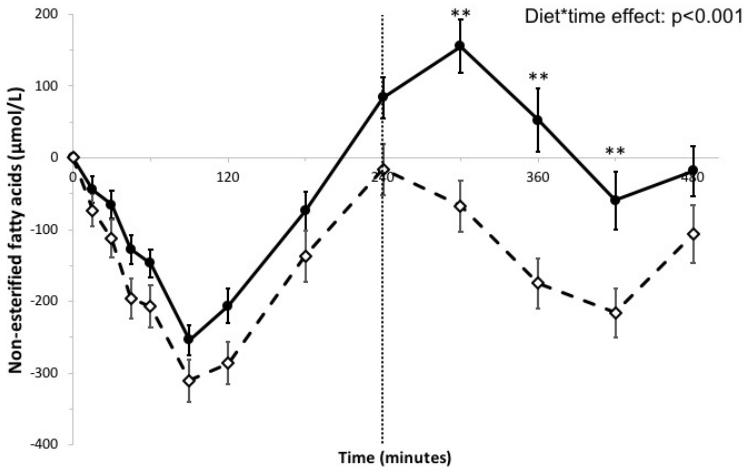

Figure 6. Postprandial changes in non-esterified fatty acids (NEFA; µmol/L) over time after meals rich in palmitic acid (•) or stearic acid (◊). NEFA concentrations were measured at baseline, and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes after meal intake. After 240 minutes, a second meal was consumed that was similar to the first meal. N=32. A significant diet*time interaction was observed (p<0.001).

Discussion

Results of this double-blind randomized study indicate that fats rich in palmitic acid or stearic acid, the two major saturated fatty acids in most Western diets, can differentially affect postprandial lipemia and possibly postprandial glycemia. Postprandial effects were studied after intakes of two consecutive mixed meals high in palmitic acid- or stearic acid-rich fat blends and tests were performed after subjects had consumed for 4 weeks diets rich in the corresponding fatty acid.

Postprandial lipemia

Postprandial lipemia was decreased after intake of the stearic acid-rich meals compared with the palmitic acid-rich meals, as indicated by lower TAG and apoB48 concentrations. Differences between the meals, as indicated by the iAUCs, were in particular evident after the first meal. Earlier studies on postprandial lipid responses between palmitic acid and stearic acid-rich meals were not consistent. In most studies [7-11], no clear differences were observed, although in one study lower TAG concentrations were reported three hours after intake of the meal rich in stearic acid [9]. In two other studies, however, lower TAG concentrations were observed after intake of a stearic acid-rich meal (lard) as compared with a palmitic acid-rich meal (palm olein) [5, 6]. These lower TAG concentrations – which agree with our findings – may relate to the physical characteristics of the fat sources used, especially to those of stearic acid, and not by the fatty-acid composition per se. It has been suggested that postprandial lipemia is attenuated if the fat is not fully liquid at 37°C [6, 18]. Indeed, lard had a higher percentage of solids at 37°C than palm olein [6] and in our study the stearic-acid fat blend also had more solids at 37°C than the palmitic-acid blend. We also observed a lower apoB48 response after intake of the stearic-acid meals. As each chylomicron particle carries one apoB48, this indicates that the number of chylomicrons after stearic-acid intake was lower. In only one other study, apoB48 responses were measured and concentrations tended to be lower after lard intake than after palm-olein intake [6]. This is in line with the hypothesis that a higher solid fat content at 37°C decreases or delays the absorption rate, resulting in less formation of chylomicron particles and consequently attenuated lipemia [18].

Participants consumed a second, identical meal 4 hours after intake of the first meal. After this second meal, differences between palmitic acid and stearic acid on postprandial lipemia were less pronounced. As TAG concentrations were still increasing 4 hours after the first meal, it can be speculated that not all the fat was absorbed, thereby increasing variability in responses and masking possible differences between the two saturated fatty acids after the second meal.

Irrespective of the fatty-acid composition of the meals, serum TAG concentrations already peaked 1 hour after the second meal and then started to decrease, while TAG concentrations

increased for up to 4 hours after the first meal. The rapid increase in TAG after the second meal may have been caused by a release of chylomicron particles that were already formed after the first meal and stored within the enterocyte [15]. This phenomenon was also observed by Baumgartner et al. [19]. In contrast, Tushuizen et al. observed a TAG peak 2 hours after a second meal [20]. However, in that study blood was sampled at 2-hour intervals and the mixed meals were provided as solid foods, while we and Baumgartner et al. [20] sampled every hour and provided the meals as a shake, which may have increased gastric emptying. Remarkably, the apoB48 peak after the second meal occurred 1 hour later than the TAG peak, while after the first meal, both apoB48 and TAG concentrations increased continuously. This was also observed by Baumgartner et al. [19] especially in participants aged between 53 and 69 years of age, which is comparable to the age of our study population. The mechanism underlying this delayed apoB48 peak compared with the TAG peak remains to be determined, but it is possible that during the first hour after the second meal, larger TAG-rich chylomicrons are secreted or that the contribution of VLDL-TAG to total TAG in the circulation is larger.

Postprandial glycemia

C-peptide concentrations were higher after the first stearic acid-rich meal and peaked earlier after the second stearic-acid rich meal. For glucose, a comparable pattern was observed, although differences did just not reach statistical significance. After both meals, postprandial NEFA suppression was more pronounced after stearic-acid intake, in particular after the second meal. Earlier studies that used a single fat-rich meal challenge did not observe any differences between palm olein or palm oil and lard on glucose [5, 13], insulin [5, 8, 13], or C-peptide [13] responses. In two studies, postprandial NEFA concentrations were lower after lard than after palm olein intake [5, 6]. Interesterified palm olein, however, had the same effect as lard, indicating that the observed differences in NEFA were most likely due to the physical characteristics of the fats rather than the fatty-acid compositions [6]. Circulating NEFAs are the resultant of adipocyte lipolysis, NEFA spillover from hydrolysis of circulating TAG-rich lipoproteins, and NEFA uptake and re-esterification [21]. We can only speculate which of these processes was mostly affected by intakes of the palmitic or stearic acid-rich fat blends. Linked to the lower TAG concentrations, the larger decrease in NEFA after stearic-acid intake may be caused by less spillover of NEFAs after hydrolysis of TAG-rich lipoproteins. In addition, increased or earlier insulin secretion after the stearic-acid meals as suggested by differences in C-peptide concentrations may have played a role.

In contrast to postprandial lipemia, differences between palmitic-acid and stearic-acid intakes on parameters related to postprandial glycemia were most pronounced after the second meal. This so-called second-meal effect is a well-known phenomenon for postprandial glucose responses, i.e. the composition of the previous meal affects the response of the subsequent meal [22], and emphasizes the importance of including second meal challenges to understand dietary effects on postprandial glycemia.

Limitations and conclusions

In the present study, blood was sampled at 15min-intervals after the first meals and at 1h-intervals after the second meals. We can therefore not exclude that the true peaks of glucose, insulin, and C-peptide after the second meals were missed. Also, palmitic acid- or stearic acid-rich meal challenges were performed after 4-week diets enriched with the corresponding fatty acid. Although this is certainly a strength as results more mimics the real-life situation, it is not known to what extent our results can be compared to acute studies.

In conclusion, our data demonstrate that the fat blend rich in stearic acid lowered postprandial lipemia as compared with the fat blend rich in palmitic acid. These effects were most pronounced after intake of the first meal and were possibly due to the higher solid fat content of the stearic acid-rich fat blends at 37°C. Differences in parameters related to postprandial glycemia were small, but seemed to be more evident after intake of the second meal, i.e. intake of the stearic acid-rich fat resulted in a faster increase in C-peptide concentrations and more pronounced decrease in NEFA concentrations. Translation of these findings into health effects on the long-term needs further study.

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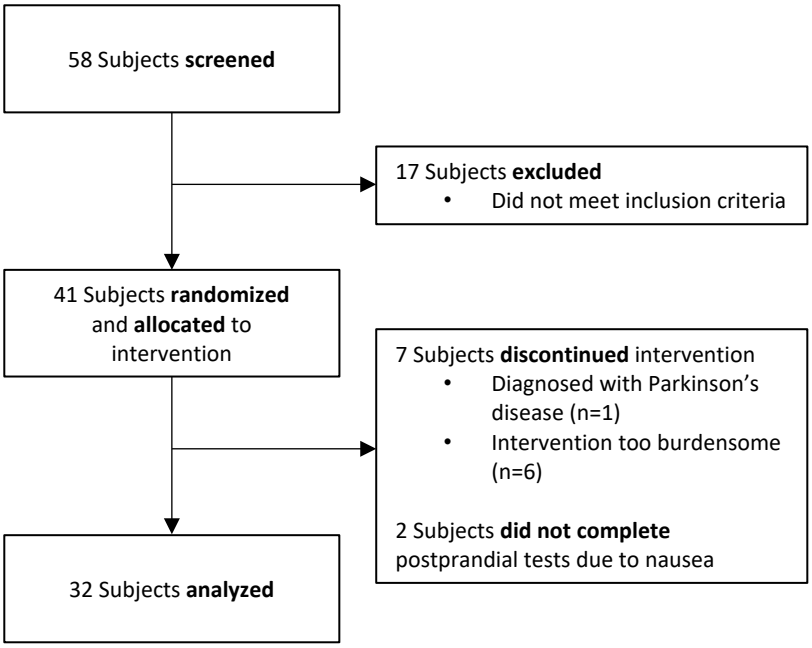
Supplemental data



Supplemental Figure 1. Flow chart of subject inclusion.

Supplemental Table 1. Fatty acid composition (w/w) of the experimental blends[1].

	C16:0-rich blend	C18:0-rich blend
Fat blend	0.9 POM/0.1 HOSO	0.92 AB/0.08 SO
SFA (g/100g fat)	50.2	50.1
C16:0	43.5	3.2
C18:0	4.6	46.1
Cis-MUFA (g/100g fat)	38.8	39.4
C18:1	38.6	39.2
Cis-PUFA (g/100g fat)	7.0	6.9
C18:2 n-6	6.7	6.1
C18:3 n-3	0.2	0.6
SMP (°C)	33.9	40.5
Solid fat 37°C (%)	1	8

[1]Amounts of fatty acids are expressed as gram fatty acids (corrected for glycerol) per 100 grams fat. Abbreviations: AB, allanblackia oil; HOSO, high-oleic sunflower oil; POM, palm oil mid-fraction; SMP, slip melting point; SO, sunflower oil.

Supplemental Table 2. Characteristics of the participants who completed the study at screening.

	All participants (n=32)	Men (n=19)	Women (n=13)
Age (y)	62 ± 5.5	61 ± 5.9	63 ± 4.9
BMI (kg/m^2)	25.6 ± 2.4	25.6 ± 1.8	25.7 ± 3.2
Total cholesterol (mmol/L)	5.64 ± 1.04	5.55 ± 1.12	5.78 ± 0.96
HDL cholesterol (mmol/L)	1.66 ± 0.39	1.53 ± 0.35	1.84 ± 0.40
Total:HDL cholesterol	3.6 ± 1.1	3.8 ± 1.1	3.3 ± 1.0
Triacylglycerol (mmol/L)	1.14 ± 0.54	1.28 ± 0.63	0.95 ± 0.27
Hb1Ac (mmol/mol)	37.5 ± 3.9	37.4 ± 4.6	37.7 ± 2.7

[1]Values are means ± SD. HbA1c, glycosylated hemoglobin A1c.

Supplemental Table 3. Postprandial responses (iAUCs) and maximal increases of triacylglycerols (TAG) after meals rich in palmitic acid or stearic acid. After 240 minutes, a second meal was consumed that was similar to the first meal.

TAG	Palmitic acid	Stearic acid	Difference C18:0-C16:0	
	LSM 95% CI	LSM 95% CI	LSM 95% CI	P-value
$iAUC_{0\text{-}8h}$ (mmol/(L*480 min))	349 289 to 409	263 203 to 323	-86 -137 to -35	0.002
$iAUC_{0\text{-}4h}$ (mmol/(L*240 min))	68 54 to 83	49 35 to 63	-19 -33 to -6	0.007
$iAUC_{4\text{-}8h}$ (mmol/(L*240 min))	107 80 to 134	86 59 to 113	-20 -47 to 6	0.127
$TAG_{max0\text{-}8h}$ (mmol/L)	1.77 1.50 to 2.04	1.41 1.15 to 1.68	-0.36 -0.58 to -0.13	0.003
$TAG_{max0\text{-}4h}$ (mmol/L)	0.80 0.66 to 0.94	0.60 0.46 to 0.74	-0.20 -0.35 to -0.06	0.007
$TAG_{max4\text{-}8h}$ (mmol/L)	0.97 0.80 to 1.14	0.82 0.65 to 0.99	-0.15 -0.32 to 0.02	0.079

Values are shown as least squared mean (LSM) with 95% confidence interval (CI). $iAUC_{0\text{-}8h}$: incremental AUC during the postprandial follow-up (0-8 hours); $iAUC_{0\text{-}4h}$= incremental AUC after the 1st meal (0-4 hours); iAUC4-8h= incremental AUC after the 2nd meal (4-8 hours). Differences in iAUCs were tested using linear mixed models. N=32.

Supplemental Table 4. Postprandial responses (iAUCs) and maximal increases of apolipoprotein B48 (apoB48) after meals rich in palmitic acid or stearic acid. After 240 minutes, a second meal was consumed that was similar to the first meal.

ApoB48	Palmitic acid	Stearic acid	Difference C18:0-C16:0	
	LSM 95% CI	LSM 95% CI	LSM 95% CI	P-value
$iAUC_{0\text{-}8h}$ (mg/(L*480 min))	1472 1278 to 1666	1187 993 to 1381	-285 -489 to -81	0.008
$iAUC_{0\text{-}4h}$ (mg/(L*240 min))	390 332 to 448	310 252 to 367	-81 -141 to -21	0.010
$iAUC_{4\text{-}8h}$ (mg/(L*240 min))	512 408 to 616	452 347 to 556	-61 -193 to 71	0.355
$ApoB48_{max0\text{-}8h}$ (mg/L)	5.96 5.15 to 6.78	5.12 4.30 to 5.93	-0.85 -1.63 to -0.69	0.034
$ApoB48_{max0\text{-}4h}$ (mg/L)	3.03 2.57 to 3.48	2.47 2.01 to 2.93	-0.56 -1.11 to -0.05	0.048
$ApoB48_{max4\text{-}8h}$ (mg/L)	3.53 2.84 to 4.23	3.29 2.60 to 3.99	-0.24 -1.14 to 0.66	0.585

Values are shown as least squared mean (LSM) with 95% confidence interval (CI). $iAUC_{0\text{-}8h}$: incremental AUC during the postprandial follow-up (0-8 hours); $iAUC_{0\text{-}4h}$= incremental AUC after the 1st meal (0-4 hours); iAUC4-8h= incremental AUC after the 2nd meal (4-8 hours). Differences in iAUCs were tested using linear mixed models. N=32.

Supplemental Table 5. Postprandial responses (iAUCs) and maximal increases of glucose after meals rich in palmitic acid or stearic acid. After 240 minutes, a second meal was consumed that was similar to the first meal.

Glucose	Palmitic acid	Stearic acid	Difference C18:0-C16:0	
	LSM 95% CI	LSM 95% CI	LSM 95% CI	P-value
$iAUC_{0\text{-}8h}$ (mmol/(L*480 min))	220 170 to 271	202 152 to 253	-18 -60 to 23	0.375
$iAUC_{0\text{-}4h}$ (mmol/(L*240 min))	68.7 50.2 to 87.2	76.3 57.8 to 94.8	7.6 -9.2 to 24.5	0.362
$iAUC_{4\text{-}8h}$ (mmol/(L*240 min))	232 183 to 282	200 150 to 249	-33 -72 to 6	0.095
$Glucose_{max0\text{-}8h}$ (mmol/L)	2.43 2.10 to 2.76	2.46 2.12 to 2.79	0.03 -0.32 to 0.37	0.876
$Glucose_{max0\text{-}4h}$ (mmol/L)	1.90 1.63 to 2.18	2.19 1.92 to 2.46	0.29 -0.01 to 0.59	0.059
$Glucose_{max4\text{-}8h}$ (mmol/L)	2.50 2.07 to 2.93	2.11 1.68 to 2.54	-0.39 -0.80 to 0.02	0.064

Values are shown as least squared mean (LSM) with 95% confidence interval (CI). $iAUC_{0\text{-}8h}$: incremental AUC during the postprandial follow-up (0-8 hours); $iAUC_{0\text{-}4h}$= incremental AUC after the 1st meal (0-4 hours); iAUC4-8h= incremental AUC after the 2nd meal (4-8 hours). Differences in iAUCs were tested using linear mixed models. N=32.

Supplemental Table 6. Postprandial responses (iAUCs) and maximal increases of insulin after meals rich in palmitic acid or stearic acid. After 240 minutes, a second meal was consumed that was similar to the first meal.

Insulin	Palmitic acid	Stearic acid	Difference C18:0-C16:0	
	LSM 95% CI	LSM 95% CI	LSM 95% CI	P-value
$iAUC_{0\text{-}8h}$ (μU/(mL*480 min))	7977 6300 to 9654	7622 5945 to 9298	-356 -1213 to 502	0.404
$iAUC_{0\text{-}4h}$ (μU/(mL*240 min))	4423 3401 to 5446	4636 3613 to 5659	212 -280 to 705	0.385
$iAUC_{4\text{-}8h}$ (μU/(mL*240 min))	4085 3296 to 4874	3417 2628 to 4206	-668 -1376 to 41	0.064
$Insulin_{max0\text{-}8h}$ (μU/mL)	79.9 62.9 to 96.9	84.2 67.3 to 101	4.4 -8.2 to 16.9	0.483
$Insulin_{max0\text{-}4h}$ (μU/mL)	79.1 62.1 to 96.2	84.2 67.2 to 101	5.1 -7.7 to 18.0	0.422
$Insulin_{max4\text{-}8h}$ (μU/mL)	43.9 33.9 to 53.8	36.4 26.5 to 46.4	-7.4 -16.8 to 1.9	0.115

Values are shown as least squared mean (LSM) with 95% confidence interval (CI). $iAUC_{0\text{-}8h}$: incremental AUC during the postprandial follow-up (0-8 hours); $iAUC_{0\text{-}4h}$= incremental AUC after the 1st meal (0-4 hours); iAUC4-8h= incremental AUC after the 2nd meal (4-8 hours). Differences in iAUCs were tested using linear mixed models. N=32.

Supplemental Table 7. Postprandial responses (iAUCs) and maximal increases of C-peptide after meals rich in palmitic acid or stearic acid. After 240 minutes, a second meal was consumed that was similar to the first meal.

C-peptide	Palmitic acid	Stearic acid	Difference C18:0-C16:0	
	LSM 95% CI	LSM 95% CI	LSM 95% CI	P-value
$iAUC_{0\text{-}8h}$ (mg/(mL*480 min))	970 845 to 1095	959 834 to 1084	-11 -114 to 92	0.825
$iAUC_{0\text{-}4h}$ (mg/(mL*240 min))	452 385 to 520	471 404 to 539	19 -24 to 62	0.379
$iAUC_{4\text{-}8h}$ (mg/(mL*240 min))	582 520 to 644	537 475 to 599	-45 -109 to 19	0.162
$C\text{-}peptide_{max0\text{-}8h}$ (ng/mL)	6.07 5.22 to 6.92	6.20 5.36 to 7.05	0.13 -0.61 to 0.87	0.717
$C\text{-}peptide_{max0\text{-}4h}$ (ng/mL)	5.84 4.99 to 6.69	6.11 5.26 to 6.96	0.27 -0.49 to 1.02	0.472
$C\text{-}peptide_{max4\text{-}8h}$ (ng/mL)	5.02 4.43 to 5.62	4.66 4.07 to 5.25	-0.36 -0.94 to 0.22	0.213

Values are shown as least squared mean (LSM) with 95% confidence interval (CI). $iAUC_{0\text{-}8h}$: incremental AUC during the postprandial follow-up (0-8 hours); $iAUC_{0\text{-}4h}$= incremental AUC after the 1st meal (0-4 hours); iAUC4-8h= incremental AUC after the 2nd meal (4-8 hours). Differences in iAUCs were tested using linear mixed models. N=32.

Supplemental Table 8. Postprandial responses (iAUCs) and maximal increases of non-esterified fatty acids (NEFA) after meals rich in palmitic acid or stearic acid. After 240 minutes, a second meal was consumed that was similar to the first meal.

NEFA	Palmitic acid	Stearic acid	Difference C18:0-C16:0	
	LSM 95% CI	LSM 95% CI	LSM 95% CI	P-value
$dAUC_{0\text{-}8h}$ (mmol/(L*480 min))	46.5 27.7 to 65.4	83.4 64.5 to 102	36.9 12.3 to 61.4	0.005
$dAUC_{0\text{-}4h}$ (mmol/(L*240 min))	31.0 21.6 to 40.3	45.2 35.9 to 54.6	14.3 1.9 to 26.6	0.025
$dAUC_{4\text{-}8h}$ (mmol/(L*240 min))	23.2 14.7 to 31.6	31.0 22.5 to 39.4	7.8 2.5 to 13.1	0.005
$NEFA_{max0\text{-}8h}$ (μmol/L)	268 217 to 318	340 290 to 391	73 10 to 134	0.026
$NEFA_{max0\text{-}4h}$ (μmol/L)	263 214 to 313	329 279 to 378	65 -1 to 131	0.054
$NEFA_{max4\text{-}8h}$ (μmol/L)	232 172 to 292	246 186 to 306	14 -28 to 55	0.499

Values are shown as least squared mean (LSM) with 95% confidence interval (CI). $dAUC_{0\text{-}8h}$: decremental AUC during the postprandial follow-up (0-8 hours); $dAUC_{0\text{-}4h}$= decremental AUC after the 1st meal (0-4 hours); dAUC4-8h= decremental AUC after the 2nd meal (4-8 hours). Differences in iAUCs were tested using linear mixed models. N=32.

CHAPTER 5

Effects of dietary palmitic acid and stearic acid on lipoprotein subfractions, ABCA1-mediated cholesterol efflux and apoA-I secretion in healthy men and postmenopausal women

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To be submitted

Abstract

Introduction: The commonly consumed saturated fatty acids palmitic acid and stearic acid differently affect fasted LDL-cholesterol, HDL-cholesterol and apoA-I concentrations. However, how different LDL and HDL subfractions as well as apoA-I secretion are affected is unclear. In addition, it is unknown how this translates into the cholesterol efflux capacity of HDL particles mediated via ATP-binding cassette transporter A1 (ABCA1-mediated CEC). Therefore, effects of 4-week diets rich in palmitic acid or stearic acid on fasting lipoprotein subfractions, and fasting and postprandial apoA-I secretion and ABCA1-mediated CEC were compared.

Methods: Twenty men and 14 postmenopausal women completed this randomized, crossover, dietary intervention study. Cholesterol and triacylglycerol (TAG) concentrations within different lipoprotein subfractions were quantified using nuclear magnetic resonance (NMR). Pro-apoA-I was measured as marker for apoA-I secretion and ABCA1-mediated CEC was measured from pCPT-cAMP treated J774 macrophages to apolipoprotein B-depleted serum.

Results: The stearic-acid diet significantly lowered cholesterol concentrations in LDL10 and LDL11 ($p<0.01$), HDL16 and HDL17 ($p<0.05$), and increased VLDL-cholesterol in VLDL03 and VLDL05 ($p<0.05$). TAG-concentrations were increased in LDL08 and LDL09 ($P<0.05$), HDL17 and HDL 18 ($p<0.05$), and VLDL05 ($p<0.05$) as well as VLDL06 and VLDL07 ($p<0.01$). Fasting apoA-I concentrations were lower on the stearic-acid diet ($p<0.01$), but no differences were observed between palmitic acid and stearic acid in fasting and postprandial pro-apoA-I concentrations as well as ABCA1-mediated CEC.

Conclusions: These findings indicate that palmitic-acid and stearic-acid diets differently affect cholesterol and TAG concentrations in different subfractions of VLDL, LDL and HDL. However, palmitic acid and stearic acid have comparable effects on fasting and postprandial ABCA1-mediated CEC as well as pro-apoA-I concentrations, even though fasting apoA-I concentrations are lower on a stearic-acid diet. These findings suggest that effects of palmitic acid versus stearic acid on lipoprotein metabolism go beyond those on LDL-cholesterol.

Introduction

Many studies have shown that dietary stearic acid (C18:0) lowers concentrations of serum LDL- and HDL-cholesterol as well as apolipoprotein A-I (apoA-I) compared with palmitic acid (C16:0) [1]. Lipoproteins, however, vary in size, density, and composition, which determines their functional and/or pathological characteristics. For LDL, particularly small-dense LDL (sdLDL) particles are considered to be atherogenic [2]. For HDL, the heterogeneous subfractions differently contribute to cholesterol efflux [3], a key feature in the reverse cholesterol transport pathway. It has been shown that particularly the smaller HDL subfractions, pre-β-HDL and HDL_3, interact with the ATP-binding cassette transporter A1 (ABCA1) receptor on macrophages to remove cholesterol [4, 5]. An increased HDL-mediated cholesterol efflux via ABCA1 is associated with a lower risk of coronary heart disease (CHD) [6]. ApoA-I, a major structural and functional protein on HDL particles, plays a crucial role in ABCA1-mediated cholesterol efflux. Thus, increasing de novo production of apoA-I may be a promising strategy to reduce CHD risk. ApoA-I is produced as a pre-pro-protein in hepatocytes and enterocytes, and secreted as pro-apoA-I [7]. Pro-apoA-I can therefore be regarded as a measure of de novo apoA-I secretion [8].

It is unclear if the commonly consumed saturated fatty acids (SFAs) palmitic acid and stearic acid differently affect lipoprotein subfractions and secretion of apoA-I, and if these changes relate to ABCA1-mediated cholesterol efflux capacity (CEC) of HDL particles. Besides, meals high in saturated fat may enhance postprandial CEC and plasma apoA-I [9]. If specific fatty acids such as palmitic acid and stearic acid have different effects on postprandial ABCA1-mediated CEC and apoA-I secretion has not been studied before. Therefore, we have studied effects on lipoprotein subfractions, apoA-I secretion and ABCA1-mediated CEC in fasting conditions after 4-week diets rich in either palmitic acid or stearic acid as well as effects on apoA-I secretion and ABCA1-mediated CEC after a meal challenge at the end of both dietary periods.

Methods

A double-blind, randomized, crossover study was performed consisting of two 4-week dietary intervention periods during which participants consumed products enriched with either palmitic acid (C16:0) or stearic acid (C18:0)-rich fat blends. Dietary intervention periods were separated by a wash-out period of at least four weeks in which participants consumed their habitual diets. Participants were stratified for gender before randomization. At the end of each intervention period, participants consumed a mixed meal high in either the palmitic acid- or stearic acid-rich fat blend. Four hours after the first meal, participants received a second, identical meal to induce a second-meal effect. The protocol was approved by Medical Ethical Committee of the MUMC+ and the study was registered at ClinicalTrials.gov with identifier NCT02835651. Effects of the 4-week diets on fasting cardiometabolic risk markers have been described previously (**Chapter 3**) as well as effects on postprandial lipemia and glycemia **(Chapter 4**).

Participants

Details on participant recruitment and inclusion criteria have been described previously (**Chapters 3 and 4**). Briefly, 41 normolipidemic and non-diabetic men (N=24) and postmenopausal women (N=17) between 45 and 70 years of age and with a BMI between 18 and 30 kg/m^2 were included. All participants gave their written informed consent before entering the study.

Intervention diets

During the 4-week intervention periods, participants consumed daily experimental foods rich in either a palmitic acid-rich or stearic acid-rich fat blends (Unilever R&D; Vlaardingen, the Netherlands; **Supplemental Table 1**). Experimental foods – buns, cookies, and lemon curd – were made especially for this study by the bakery department of the Hotelschool Hasselt in Belgium. The amount of experimental foods that participants consumed daily was based on the daily energy requirement calculated before the start of the intervention periods. Intervention diets consisted of 35% daily energy (en%) as fat, of which 16 en% was provided by either the palmitic acid- or stearic acid-rich fat blend, incorporated into the experimental foods. To keep energy and nutrient intakes comparable – except for a targeted difference of 6 en% between stearic acid and palmitic acid intake – between the two intervention periods, dietary guidelines were provided by a dietician. Participants recorded daily their foods choices in a food diary and completed at the end of each intervention period a food frequency questionnaire (FFQ) to estimate total energy and nutrient intakes using the Dutch food composition table (NEVO).

Participants visited the metabolic research unit Maastricht (MRUM) at days 0, 14, 25, and 28, and blood was sampled at days 0, 25 and 28. Here, only results of days 0 and 28 are presented, as not all measurements could be performed in all samples. For earlier

published values of the end of the dietary intervention periods, means of days 25 and 28 were used (Chapter 3), which explains why end values in the present paper (day 28) are slightly different from earlier published values.

Postprandial test

At the end of each intervention period, participants followed an 8-hour postprandial test during which they consumed two consecutive, identical mixed meals provided as a shake high in either the palmitic acid- or stearic acid-rich fat blend (Table 1). Of the 50 grams of fat provided by the shakes, 46.6 grams originated from the fat blend that participants also received during the preceding four weeks. Four hours after the first meal, participants consumed the second meal. During the test day, multiple blood samples were obtained but here only results of the baseline (T0) sample and the samples 4 and 8 hours after consumption of the first meals (T4 and T8 respectively) are reported. The second meal was consumed immediately after the blood sample obtained at T4. Participants were only allowed to drink water during the entire postprandial test.

Table 1. Nutrient composition of the standardized shakes provided for breakfast and lunch.

	Palmitic acid-rich shake	Stearic acid-rich shake
Energy (kcal)	697.4	697.4
Carbohydrates (en%)	30.8 (54g)	30.8 (54g)
Protein (en%)	4.8 (5g)	4.8 (5g)
Fat (en%)	64.5 (50g)	64.5 (50g)
SFA (en%)	33.0	33.0
C16:0 (en%)	28.3 (22g)	3.1 (2.5g)
C18:0 (en%)	3.2 (2.4g)	29.2 (22.6g)
MUFA (en%)	26.0	26.4
C18:1 (en%)	25.7	26.1
PUFA (en%)	4.7	4.7
C18:3 n-3 (en%)	0.1	0.4
Cholesterol (mg)	120	120
Fiber (g)	1.28	1.28

SFA: saturated fatty acids, MUFA: cis-monounsaturated fatty acids, PUFA: cis-polyunsaturated fatty acids.

Blood collection and biochemical analyses

Blood was sampled in serum separator vacutainer tubes (Becton, Dickinson and company, NJ, USA) for analyses of ABCA1-mediated CEC, apoA-I, pro-apoA-I, and the composition of lipoprotein subfractions. After sampling, serum tubes were allowed to clot for at least 30 minutes at room temperature and subsequently centrifuged at 1300×g for 15 minutes at 20°C. Aliquots of serum were snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Ex vivo ABCA1-mediated CEC was measured at day 0, and at baseline (T0), T4, and T8 of day 28. Detailed information of the used method to measure CEC has been described previously (Chapter 3). pCPT-cAMP treated J774 macrophages were used as cholesterol donor and apoB-depleted serum from a participant as cholesterol acceptor. To normalize CEC values, a pooled serum sample from healthy volunteers was included on each plate. The CEC value of this pool was set at 100% and participant values were expressed relative to those of the pool (% pool). At the same time points, apoA-I concentrations were measured immunoturbidimetrically (Horiba ABX, Montpellier, France) and pro-apoA-I concentrations were measured with an ELISA, as described (Chapter 6).

Cholesterol and triacylglycerol concentrations within lipoprotein subfractions were measured using nuclear magnetic resonance (NMR) spectroscopy [10]. One baseline (day 0) sample of one subject was missing. Cholesterol and triacylglycerol concentrations were measured in 15 lipoprotein subfractions, i.e. 2 chylomicron subfractions (CM1: >90 nm, CM2: 78 nm), 5 VLDL subfractions (VLDL03: 64.0 nm, VLDL04: 53.6 nm, VLDL05: 44.5 nm, VLDL06: 36.8 nm, VLDL07: 31.3 nm), 4 LDL subfractions (LDL08: 28.6 nm, LDL09: 25.5 nm, LDL10: 23.0 nm, LDL11: 20.7 nm,) and 4 HDL subfractions (HDL15: 13.5 nm, HDL16: 12.1 nm, HDL17: 10.9 nm, HDL18: 9.8 nm). In addition, concentrations were measured within total VLDL (particle size: 30-80 nm), total LDL (16-30 nm), and total HDL (8-16 nm) [11].

Statistical analyses

Date are reported as least squared mean (LSM) with 95% confidence interval (CI) unless otherwise indicated. Effects of diets rich in palmitic acid or stearic acid were compared using linear mixed models with participants as random factor, period and diet as fixed factors, and baseline concentrations of the corresponding period (day 0) as well as average baseline concentrations of both periods (average day 0) as covariates. Postprandial changes over time were compared separately for meals rich in palmitic acid or stearic acid using linear mixed models with subjects as between subject variable and time as fixed factor. Postprandial effects of the palmitic acid-rich or stearic acid-rich meals were compared using linear mixed models with subjects as between subject variable, baseline concentrations (T0) as covariate, and period, meal, time, meal*time, and baseline (T0) as fixed factors. If the meal*time interaction term did not reach statistical significance, it was omitted from the model. Pairwise comparisons were performed with a Bonferroni post-hoc analysis. Differences are reported as least squared means (LSM) with 95% confidence interval (CI) obtained from linear mixed models. Results were considered statistically significant when the p-value was < 0.05. Statistical analyses were performed using IBM SPSS Statistics for Mac, version 24.0 (IBM Corp., Armonk, NY, USA).

Results

Twenty men and 14 postmenopausal women completed the study. As one man and one woman did not complete one or both postprandial tests due to nausea, only 32 participants were included in the postprandial analyses.

Fasting cholesterol concentrations within lipoprotein subfractions

Cholesterol concentrations in chylomicron (CM)01 and CM02 particles did not differ significantly between diets (Table 2). At the end of the stearic-acid diet, cholesterol concentrations were increased in the total very-low density lipoprotein (VLDL) fraction (+0.06 mmol/L; p=0.021) compared with the palmitic-acid diet, mainly in the VLDL subfractions VLDL03 (+0.01 mmol/L; p=0.047) and VLDL05 (+0.02 mmol/L; p=0.035). Lower cholesterol concentrations were observed in total LDL (-0.12 mmol/L; p=0.007) and in all subfractions of LDL on the stearic-acid diet, although the difference in the larger LDL08 and LDL09 subfractions did not reach statistical significance (p=0.056 and p=0.050 respectively). The decrease in the smaller LDL subfractions LDL10 and LDL11 was -0.03 mmol/L (p=0.007) and -0.01 mmol/L (p=0.005) respectively. In addition, HDL-cholesterol concentrations were lower on the stearic-acid diet in the HDL16 (-0.02 mmol/L; p=0.025) and HDL17 (-0.02 mmol/L; p=0.030) subfractions as well as in the total HDL fraction (-0.06 mmol/L; p=0.005).

Fasting triacylglycerol concentrations within lipoprotein subfractions

TAG concentrations in CM particles were not changed, but increased on the stearic-acid diet in the smaller VLDL subfractions VLDL05 (+0.23 mmol/L*10; p=0.031), VLDL06 (+0.13 mmol/L*10; p=0.009), and VLDL07 (+0.07 mmol/L*10; p=<0.001), but not in the total VLDL fraction (p=0.134; Table 3). In the total LDL fraction, TAG concentrations were higher on the stearic-acid diet (+0.15 mmol/L*10; p=0.009), which was due to the larger LDL08 (+0.07 mmol/L*10; p=0.002), and LDL09 (+0.04 mmol/L*10; p=0.042) subfractions. TAG in the total HDL fraction was not different (p=0.360), although TAG concentrations were higher in the smaller HDL17 (+0.04 mmol/L*10; p=0.027) and HDL18 (+0.03 mmol/L*10; p=0.033) subfractions on the stearic-acid diet.

Fasting cholesterol efflux capacity, apoA-I concentrations, and apoA-I secretion

ABCA1-mediated CEC in fasting conditions did not differ significantly between the diets (p=0.086; Table 4). ApoA-I concentrations were 0.04 g/L lower at the end of the stearic-acid diet (p=0.009), but pro-apoA-I concentrations were comparable (p=0.975).

Postprandial cholesterol efflux capacity, apoA-I concentrations, and apoA-I secretion

Compared to baseline, no postprandial changes in ABCA1-mediated CEC were observed after intake of the stearic acid-rich meals (time-effect: p=0.419) or the palmitic acid-rich meals (time-effect: p=0.091; **Figure 1**). Changes over time were also not different between palmitic-acid and stearic-acid intakes (p=0.782 for the meal*time interaction), and no diet-effects were observed (p=0.818).

ApoA-I concentrations increased compared with baseline after intake of the stearic acid-rich meals (**Figure 2**), i.e. concentrations were 0.03 g/L higher at 4 hours (95% CI: 0.01 to 0.05 g/L; p=0.005) and at 8 hours postprandially (95% CI: 0.01 to 0.05 g/L; p=0.001). Concentrations were comparable between T4 and T8. Similar results were seen after intake of the palmitic acid-rich meal (**Figure 2**). No meal*time effect was observed (p=0.796), but apoA1 concentrations were on average 0.04 g/L lower after stearic-acid intake than after palmitic-acid intake (meal-effect: 95% CI: -0.07 to -0.01; p=0.004).

Compared with baseline, postprandial pro-apoA-I concentrations after stearic-acid intakes were 4.12 mg/L lower at 4 hours (95% CI: -7.06 to -1.17 mg/L; p=0.003), but no difference was observed at 8 hours (p=0.976; **Figure 3**). The differences in concentrations of -2.93 mg/L between T4 and T8 did just not reach statistical significance (95% CI: -5.87 to 0.02 mg/L; p=0.052). After intake of the palmitic acid-rich meals, concentrations were 2.90 mg/L lower at T4 (95% CI: -5.09 to -0.72; p=0.005), while concentrations at T8 did not differ from those at baseline. At T4, concentrations were 2.96 mg/L lower than at T8 (95% CI: -5.15 to -0.77 mg/L; p=0.004; **Figure 3**). For pro-apoA-I, no meal*time effect (p=0.985) or meal-effect (p=0.206) was observed.

Table 2. Fasted cholesterol concentrations (mmol/L) within lipoprotein subfractions on diets rich in stearic acid versus palmitic acid.

Cholesterol (mmol/L)	Diet	Means ± SD[1]		Difference with 95% CI[2]	p-value
		Baseline	End		
CM01	C16:0	0.04 ± 0.05	0.03 ± 0.03	0.01 (0.00; 0.02)	0.153
	C18:0	0.03 ± 0.03	0.04 ± 0.04		
CM02	C16:0	0.03 ± 0.03	0.02 ± 0.02	0.00 (0.00; 0.01)	0.110
	C18:0	0.02 ± 0.02	0.03 ± 0.02		
Total VLDL	C16:0	1.22 ± 0.50	1.21 ± 0.39	0.06 (0.01; 0.11)	0.021
	C18:0	1.23 ± 0.37	1.26 ± 0.40		
VLDL03	C16:0	0.09 ± 0.08	0.08 ± 0.05	0.01 (0.00; 0.02)	0.047
	C18:0	0.08 ± 0.05	0.09 ± 0.06		
VLDL04	C16:0	0.13 ± 0.10	0.11 ± 0.07	0.02 (0.00; 0.04)	0.059
	C18:0	0.12 ± 0.08	0.13 ± 0.08		
VLDL05	C16:0	0.44 ± 0.21	0.45 ± 0.16	0.02 (0.00; 0.04)	0.035
	C18:0	0.44 ± 0.15	0.46 ± 0.16		
VLDL06	C16:0	0.26 ± 0.12	0.26 ± 0.10	0.01 (-0.01; 0.02)	0.328
	C18:0	0.26 ± 0.09	0.26 ± 0.10		
VLDL07	C16:0	0.25 ± 0.07	0.26 ± 0.07	0.01 (0.00; 0.02)	0.173
	C18:0	0.26 ± 0.07	0.26 ± 0.08		
Total LDL	C16:0	2.87 ± 0.77	2.86 ± 0.77	-0.12 (-0.20; -0.03)	0.007
	C18:0	2.93 ± 0.69	2.74 ± 0.69		
LDL08	C16:0	0.84 ± 0.22	0.87 ± 0.21	-0.03 (-0.06; 0.00)	0.056
	C18:0	0.87 ± 0.19	0.84 ± 0.19		
LDL09	C16:0	1.20 ± 0.40	1.22 ± 0.39	-0.04 (-0.09; 0.00)	0.050
	C18:0	1.22 ± 0.38	1.77 ± 0.38		
LDL10	C16:0	0.49 ± 0.17	0.45 ± 0.16	-0.03 (-0.05; -0.01)	0.007
	C18:0	0.49 ± 0.16	0.42 ± 0.16		
LDL11	C16:0	0.16 ± 0.05	0.15 ± 0.05	-0.01 (-0.02; 0.00)	0.005
	C18:0	0.16 ± 0.05	0.14 ± 0.05		
Total HDL	C16:0	1.30 ± 0.31	1.33 ± 0.25	-0.06 (-0.10; -0.02)	0.005
	C18:0	1.31 ± 0.29	1.28 ± 0.25		
HDL15	C16:0	0.06 ± 0.04	0.06 ± 0.03	-0.00 (-0.01; 0.00)	0.259
	C18:0	0.06 ± 0.04	0.06 ± 0.03		
HDL16	C16:0	0.23 ± 0.15	0.24 ± 0.13	-0.02 (-0.03; 0.00)	0.025
	C18:0	0.24 ± 0.15	0.23 ± 0.13		
HDL17	C16:0	0.46 ± 0.08	0.48 ± 0.07	-0.02 (-0.03; 0.00)	0.030
	C18:0	0.46 ± 0.07	0.46 ± 0.08		

HDL18	C16:0	0.38 ± 0.04	0.38 ± 0.04	0.00 (-0.01; 0.01)	0.605
	C18:0	0.38 ± 0.05	0.38 ± 0.04		

[1]Baseline (day 0) and End (day 28) concentrations are expressed as unadjusted means ± SD (n=34).
[2]Differences between the palmitic acid- and stearic acid-rich diets, expressed as least squared means with 95% confidence interval (95% CI), were estimated using linear mixed models.
Particle sizes of lipoprotein subfractions: CM1: >90 nm, CM2: 78 nm, VLDL03: 64.0 nm, VLDL04: 53.6 nm, VLDL05: 44.5 nm, VLDL06: 36.8 nm, VLDL07: 31.3 nm, LDL08: 28.6 nm, LDL09: 25.5 nm, LDL10: 23.0 nm, LDL11: 20.7 nm, HDL15: 13.5 nm, HDL16: 12.1 nm, HDL17: 10.9 nm, and HDL18: 9.8 nm. In addition, concentrations within total VLDL (particle size: 30-80 nm), total LDL (16-30 nm), and total HDL (8-16 nm) were calculated.

Table 3. Fasted triacylglycerol (TAG) concentrations (mmol/L) within lipoprotein subfractions on diets rich in stearic acid versus palmitic acid.

TAG (mmol/L x 10)	Diet	Means ± SD[1]		Difference with 95% CI[2]	P-value
		Baseline	End		
CM01	C16:0	0.84 ± 0.87	0.67 ± 0.88	0.05 (-0.27; 0.36)	0.763
	C18:0	0.74 ± 0.84	0.72 ± 0.88		
CM02	C16:0	0.66 ± 0.54	0.52 ± 0.05	0.04 (-0.12; 0.19)	0.625
	C18:0	0.61 ± 0.55	0.56 ± 0.05		
Total VLDL	C16:0	8.62 ± 3.81	7.34 ± 3.47	0.66 (-0.22; 1.54)	0.134
	C18:0	8.31 ± 3.80	7.99 ± 3.75		
VLDL03	C16:0	1.50 ± 1.14	1.18 ± 0.97	0.17 (-0.18; 0.40)	0.435
	C18:0	1.39 ± 1.12	1.29 ± 1.02		
VLDL04	C16:0	2.22 ± 1.43	1.81 ± 1.22	0.17 (-0.18; 0.52)	0.321
	C18:0	2.09 ± 1.39	1.98 ± 1.26		
VLDL05	C16:0	2.61 ± 1.00	2.25 ± 0.91	0.23 (0.02; 0.44)	0.031
	C18:0	2.56 ± 1.05	2.48 ± 1.06		
VLDL06	C16:0	1.18 ± 0.41	1.10 ± 0.39	0.13 (0.04; 0.22)	0.009
	C18:0	1.21 ± 0.41	1.24 ± 0.48		
VLDL07	C16:0	0.56 ± 0.23	0.55 ± 0.17	0.07 (0.04; 0.10)	<0.001
	C18:0	0.57 ± 0.19	0.62 ± 0.22		
Total LDL	C16:0	2.73 ± 0.84	2.51 ± 0.61	0.15 (0.04; 0.26)	0.009
	C18:0	2.70 ± 0.71	2.69 ± 0.81		
LDL08	C16:0	1.03 ± 0.36	0.99 ± 0.27	0.07 (0.03; 0.12)	0.002
	C18:0	1.04 ± 0.30	1.08 ± 0.35		
LDL09	C16:0	1.06 ± 0.31	0.97 ± 0.23	0.04 (0.00; 0.09)	0.042
	C18:0	1.05 ± 0.28	1.03 ± 0.30		
LDL10	C16:0	0.44 ± 0.12	0.39 ± 0.09	0.01 (-0.01; 0.04)	0.335
	C18:0	0.43 ± 0.11	0.40 ± 0.11		
LDL11	C16:0	0.14 ± 0.04	0.13 ± 0.03	0.00 (0.00; 0.01)	0.267
	C18:0	0.14 ± 0.04	0.13 ± 0.04		

Total HDL	C16:0	1.49 ± 0.50	1.37 ± 0.37	0.05 (-0.05; 0.15)	0.360
	C18:0	1.49 ± 0.34	1.43 ± 0.44		
HDL15	C16:0	0.07 ± 0.07	0.06 ± 0.03	0.00 (0.00; 0.01)	0.086
	C18:0	0.07 ± 0.04	0.06 ± 0.04		
HDL16	C16:0	0.29 ± 0.17	0.27 ± 0.01	0.00 (-0.02; 0.03)	0.652
	C18:0	0.29 ± 0.12	0.27 ± 0.01		
HDL17	C16:0	0.51 ± 0.12	0.50 ± 0.11	0.04 (0.00; 0.07)	0.027
	C18:0	0.51 ± 0.11	0.54 ± 0.14		
HDL18	C16:0	0.37 ± 0.11	0.34 ± 0.10	0.03 (0.00; 0.06)	0.033
	C18:0	0.36 ± 0.11	0.37 ± 0.12		

[1]Baseline (day 0) and End (day 28) concentrations are expressed as unadjusted means ± SD (n=34).
[2]Differences between the palmitic acid- and stearic acid-rich diets, expressed as least squared means with 95% confidence interval (95% CI), were estimated using linear mixed models.
Particle sizes of lipoprotein subfractions: CM1: >90 nm, CM2: 78 nm, VLDL03: 64.0 nm, VLDL04: 53.6 nm, VLDL05: 44.5 nm, VLDL06: 36.8 nm, VLDL07: 31.3 nm, LDL08: 28.6 nm, LDL09: 25.5 nm, LDL10: 23.0 nm, LDL11: 20.7 nm, HDL15: 13.5 nm, HDL16: 12.1 nm, HDL17: 10.9 nm, and HDL18: 9.8 nm. In addition, concentrations within total VLDL (particle size: 30-80 nm), total LDL (16-30 nm), and total HDL (8-16 nm) were calculated.

Table 4. Fasting cholesterol efflux capacity and apoA-I secretion on diets rich in stearic acid versus palmitic acid.

		Means ± SD[1]		Difference with 95% CI[2]	P-value
	Diet	Baseline	End		
CEC (% pool)	C16:0	96.6 ± 6.4	94.3 ± 8.0	2.6 (-0.4, 5.6)	0.086
	C18:0	98.9 ± 7.0	96.8 ± 7.0		
ApoA-I (g/L)[3]	C16:0	1.52 ± 0.18	1.40 ± 0.14	-0.04 (-0.07, -0.01)	0.009
	C18:0	1.52 ± 0.17	1.36 ± 0.15		
Pro-apoA-I (mg/L)	C16:0	52.2 ± 18.6	55.1 ± 20.0	-0.04 (-2.31, 2.24)	0.975
	C18:0	51.6 ± 17.3	55.0 ± 20.8		

[1]Baseline (day 0) and End (day 28) concentrations are expressed as unadjusted means ± SD (n=34).
[2]Differences between the palmitic acid- and stearic acid-rich diets, expressed as least squared means with 95% confidence interval (95% CI), were estimated using linear mixed models.
[3]n=32. Abbreviations: ABCA1, ATP-binding cassette transporter A1; CEC, cholesterol efflux capacity

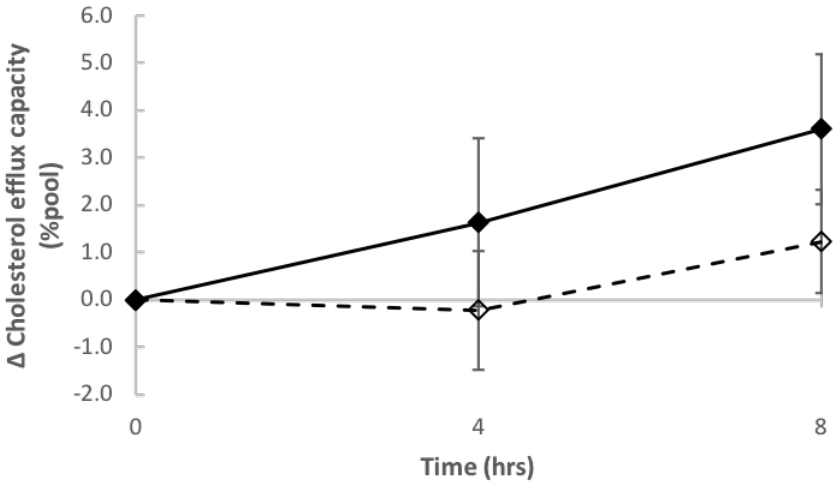

Figure 1. Postprandial changes in cholesterol efflux capacity (expressed as % compared to pool) over time after meals rich in palmitic acid (•) or stearic acid (◊). Concentrations were measured at baseline, and 4 and 8 hours after intake of the first meal. After 4 hours, a second meal was consumed that was similar to the first meal. N=32.

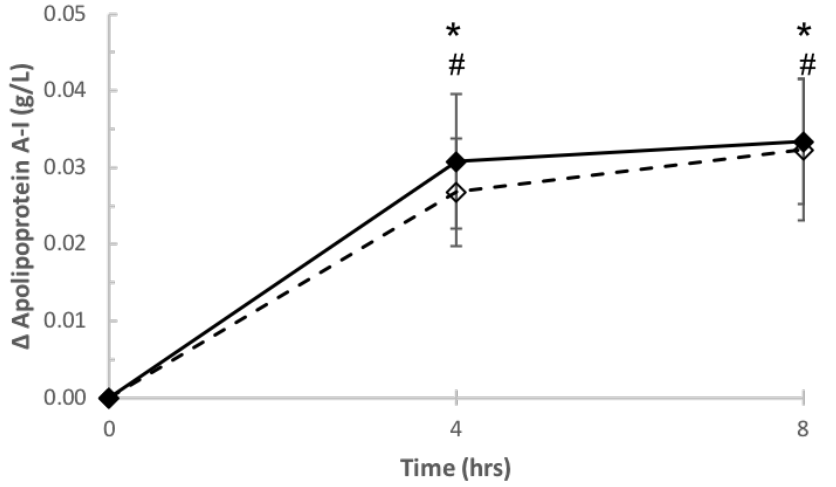

Figure 2. Postprandial changes in apolipoproteinA-I (g/L) over time after meals rich in palmitic acid (•) or stearic acid (◊). Concentrations were measured at baseline, and 4 and 8 hours after intake of the first meal. After 4 hours, a second meal was consumed that was similar to the first meal. N=32. *Significantly different compared to baseline values after the palmitic acid-rich meals. #Significantly different compared to baseline values after the stearic acid-rich meals.

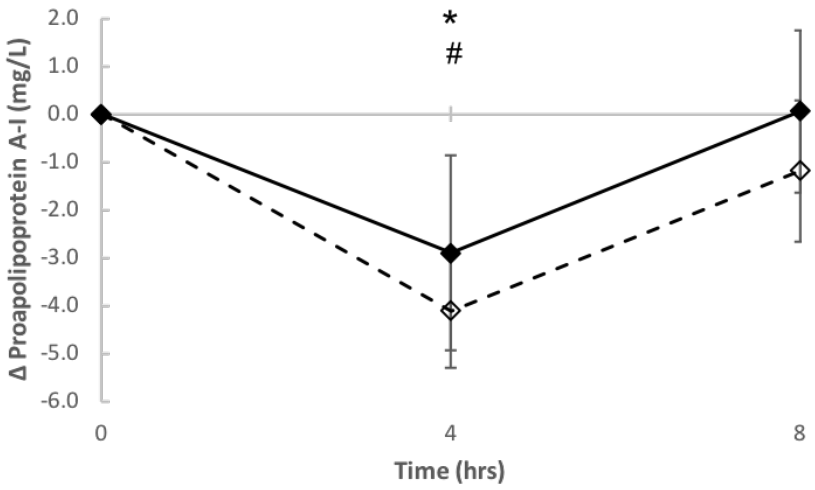

Figure 3. Postprandial changes in pro-apolipoproteinA-I (mg/L) over time after meals rich in palmitic acid (●) or stearic acid (◊). Concentrations were measured at baseline, and 4 and 8 hours after intake of the first meal. After 4 hours, a second meal was consumed that was similar to the first meal. N=32. *Significantly different compared to baseline values after the palmitic acid-rich meals. #Significantly different compared to baseline values after the stearic acid-rich meals.

Discussion

It is well-known that stearic acid lowers LDL and HDL cholesterol concentrations as compared with palmitic acid [1]. However, effects of these two SFAs on cholesterol and triacylglycerol concentrations within the different lipoprotein subfractions of LDL and HDL have been not been compared so far side-by-side. We found that stearic acid lowered LDL-cholesterol concentrations in all subfractions by approximately 5% as compared with palmitic acid, although effects were statistically more significant in the smaller, denser subfractions (LDL10 and LDL11 with mean diameters of respectively 23.0 and 20.7 nm). Several studies have now suggested that particularly sdLDL particles are atherogenic [2, 12]. Unfortunately, we could not accurately quantify lipid concentrations in these sdLDL subfractions (particle size of 18.0 to 20.5 nm when measured with NMR [13]) and the mean diameter of our smallest LDL fraction (LDL11) was 20.7 nm. Thus, whether the cholesterol content of the sdLDL subfractions was also lowered by stearic acid needs further study. For HDL, cholesterol concentrations were significantly lowered in the middle two (HDL16 and HDL17 with mean diameters of respectively 12.1 and 10.9 nm) of the four HDL subfractions on the stearic-acid diet. This suggests that the effects were subclass specific. As especially small HDL subfractions are thought to promote cholesterol efflux via the ABCA1 transporter [14], this finding may explain why ABCA1-mediated CEC of apoB-depleted serum was not significantly different between the stearic-acid diet and the palmitic-acid diet. In contrast to previous studies [15-18], we found

that total VLDL-cholesterol concentrations were increased by approximately 5% on the stearic-acid rich diet. This discrepancy may be related to different methods used to quantify VLDL-cholesterol. The observed increases in VLDL-cholesterol were mainly observed in the 3 larger VLDL subfractions (VLDL03, VLDL04, and VLDL05 with mean diameters of respectively 64.0, 53.6 and 44.5 nm).

Fasting serum total TAG concentrations were slightly, though not significantly, increased on day 28 of the stearic-acid diet (data not shown). The NMR data presented here suggests that TAG concentrations in some VLDL, LDL and HDL subfractions were increased on the stearic-acid diet, mainly in the smaller VLDL (VLDL05, VLDL06, and VLDL07), larger LDL (LDL08 and LDL09), and smaller HDL (HDL17 and HDL18) subfractions. In a recent study [19], TAG concentrations measured with NMR in all VLDL and LDL subfractions as well as in most HDL subfractions were positively associated with myocardial infarction and to a lesser extent also to ischemic stroke. In addition, HDL-TAG concentrations were higher in patients with carotid plaques, as well as in patients with type 2 diabetes or the metabolic syndrome [20]. Thus, even though stearic-acid diets may favorably affect LDL-cholesterol concentrations as compared with palmitic-acid diets, the observed increases in TAG concentrations within lipoprotein subfractions may be more unfavorable. It should be noted, that different methods exist to examine lipoprotein subfractions. As we have quantified lipid concentrations within the different subfractions, it remains to be determined whether the number of particles within the subfractions was also changed. For example, we observed an increase in cholesterol concentrations in the larger VLDL subfractions. Whether this indicates that there are more larger VLDL particles on the stearic-acid diet, and as such also results in more sdLDL particles, cannot be concluded from this data. However, Holmes et al. observed that associations between coronary diseases and cholesterol concentrations within subfractions were very similar to those of particle concentrations [19].

At the end of the intervention periods, fasting and postprandial pro-apoA-I concentrations did not differ between the palmitic acid and stearic acid diets. However, independent of the fatty-acid consumed, postprandial pro-apoA-I concentrations decreased compared to baseline 4 hours after intake of the first meal, but not after the second meal. We have previously measured pro-apoA-I concentrations after intake of a high-fat meal (Chapter 6). In that study, pro-apoA-I concentrations were increased 4 hours after meal-intake. As total fat intake of the postprandial meals in the two studies was comparable, this discrepancy may relate to the meal compositions, as the high-fat meal in our previous study provided 40 grams more carbohydrates and 14 grams more protein as compared with the current test meals. It was shown that a high-protein meal increased pro-apoA-I concentrations more than a high-fat meal (Chapter 6). In addition, the fat source used in that study was butter. Although butter contains a comparable amount of SFAs as the fat blends used in the present study, the amount of long-chain SFAs palmitic acid and stearic acid is much lower and of the short- or

medium-chain SFAs higher. Possibly long-chain SFAs affect the secretion of apoA-I differently than short- or medium-chain SFAs. Indeed, *in vitro* the HepG2 proliferator-activated receptor alpha (PPARα) – one of the regulators of apoA-I production [22, 23] – was inhibited to the same extent by addition of palmitic acid or stearic acid, while it was activated by caprylic acid (C8:0), lauric acid (C12:0), and low doses of myristic acid (C14:0) compared to basal activation [24]. Whether this also means that the long-chain SFAs palmitic acid and stearic acid inhibit apoA-I secretion compared to short- and medium-chain SFAs remains to be determined.

In contrast to pro-apoA-I, fasting apoA-I concentrations were lower on the stearic-acid diets, which agrees with other studies [1]. Nevertheless, postprandial changes in apoA-I did not depend on the amount of palmitic acid or stearic acid in the test meals, which is in line with the findings of Tholstrup et al. [25]. Surprisingly, a decrease in pro-apoA-I concentrations 4 hours after consumption of the first meal was observed, while apoA-I concentrations increased. For the first meal, it can be speculated that these findings can be explained by a higher conversion of pro-apoA-I into apoA-I. If so, then there is no obvious reason why this effect was not evident after the second meal. Alternatively, apoA-I clearance may have decreased during the postprandial phase, resulting in higher apoA-I concentrations.

Both fasting and postprandial ABCA1-mediated CEC were not differently affected by palmitic-acid or stearic-acid intakes. In addition, no significant postprandial changes over time were observed. This is not in line with previous studies, as most studies that have examined the effects of high-fat meal have shown that CEC increased 4 hours after meal consumption [9, 26-30]. The reason behind this discrepancy is unknown.

In conclusion, these findings indicate that palmitic-acid and stearic-acid diets differently affect cholesterol and triacylglycerol concentrations in different subfractions of VLDL, LDL, and HDL as measured with NMR. However, palmitic acid and stearic acid have comparable effects on fasting and postprandial ABCA1-mediated CEC as well as pro-apoA-I concentrations, even though fasting apoA-I concentrations are lower on a stearic-acid diet. These findings suggest that effects of palmitic acid versus stearic acid on lipoprotein metabolism go beyond those on LDL-cholesterol. However, future studies are needed to confirm or refute these novel findings.

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Supplemental data

Supplemental Table 1. Fatty acid composition (w/w) of the experimental blends[1].

	C16:0-rich blend	C18:0-rich blend
Fat blend	0.9 POM/0.1 HOSO	0.92 AB/0.08 SO
SFA (g/100g fat)	50.2	50.1
C16:0	43.5	3.2
C18:0	4.6	46.1
Cis-MUFA (g/100g fat)	38.8	39.4
C18:1	38.6	39.2
Cis-PUFA (g/100g fat)	7.0	6.9
C18:2 n-6	6.7	6.1
C18:3 n-3	0.2	0.6
SMP (°C)	33.9	40.5
Solid fat 37°C (%)	1	8

[1]Amounts of fatty acids are expressed as gram fatty acids (corrected for glycerol) per 100 grams fat. Abbreviations: AB, allanblackia oil; HOSO, high-oleic sunflower oil; POM, palm oil mid-fraction; SMP, slip melting point; SO, sunflower oil.

CHAPTER 6

A comparison of the postprandial effects from high-fat, high-protein or high-carbohydrate meals on ABCA1-mediated cholesterol efflux and apoA-I secretion in overweight or slightly obese men

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To be submitted

Abstract

Introduction: In the postprandial state, high-fat meals may increase the capacity of high-density lipoprotein (HDL) particles to accept cholesterol from macrophages (cholesterol efflux capacity; CEC), an emerging risk marker for the development of coronary heart disease (CHD). Postprandial effects of high-protein or high-carbohydrate meals however have not been studied. Also, effects of these macronutrients on apoA-I secretion, an important determinant of ATP-binding cassette transporter A1 (ABCA1) mediated CEC, are largely unknown. Therefore, we have compared side-by-side the effects of high-fat, high-protein, or high-carbohydrate meals on postprandial ABCA1-mediated CEC and apoA-I secretion.

Methods: Eighteen apparently healthy men (BMI 30.5 ± 2.9 kg/m^2) consumed in random order mixed meals high in either fat, carbohydrates, or proteins. Blood was sampled at baseline, and 60 and 240 minutes after meal intake to measure ABCA1-mediated CEC, and apoA-I and pro-apoA-I concentrations.

Results: None of the meals affected postprandial ABCA1-mediated CEC or apoA-I concentrations. After 240 minutes, pro-apoA-I concentrations were increased by 4.21 mg/L ($p=0.034$) after the high-fat meal and by 10.4 mg/L ($p<0.001$) after the high-protein meal, but not after the high-carbohydrate meal (3.83 mg/L; $p=0.090$). Increases after the high-protein meal were more pronounced than those after intake of the high-carbohydrate and high-fat meals ($p=0.021$ for meal*time effect).

Conclusion: Postprandial ABCA1-mediated CEC or apoA-I concentrations were not affected by intake of the high-fat, high-protein, or high-carbohydrate meals, even though the high-protein and high-fat meals increased pro-apoA-I concentrations.

Introduction

The capacity of high-density lipoprotein (HDL) particles to accept cholesterol from macrophages (cholesterol efflux capacity; CEC) is an emerging risk marker for the development of coronary heart disease (CHD). In fact, HDL-mediated cholesterol efflux is a key process of the reverse cholesterol transport pathway and inversely associated with atherosclerosis development [1] and cardiovascular events [2]. Dietary intake may affect CEC [3, 4] and as we spent a large part of the day in a postprandial state, understanding effects of nutrients on postprandial HDL-mediated cholesterol efflux is important. Many studies have now shown that a high-fat meal increases postprandial CEC compared to fasting values [5-11]. Whether meals high in carbohydrates or proteins affect postprandial CEC has however not been studied. Moreover, the mechanism underlying changes in postprandial CEC after a high-fat meal is not well understood. Modification of HDL profiles may be involved, as postprandial lipemia was associated with more plasma HDL_2 and less HDL_3 as well as an increased capacity of HDL_2 particles to accept cholesterol [8]. By comparing the effects of fat, carbohydrate and protein on postprandial HDL-mediated cholesterol efflux, we get more insight into underlying mechanisms, i.e. if changes in postprandial HDL-mediated cholesterol efflux are related to changes in triacylglycerols, glucose, or insulin.

Cholesterol efflux can be mediated via different pathways. However, cholesterol efflux mediated via the ATP-binding cassette transporter (ABC) A1 (ABCA1-mediated CEC) is the most important in the context of atherosclerosis development, as it is the predominant cholesterol efflux pathway from lipid-loaded macrophages to HDL [12]. For ABCA1-mediated CEC, interaction with apolipoprotein (apo) A-I – the most abundant protein on HDL particles – is crucial. ApoA-I is produced in hepatocytes and enterocytes as pre-pro-apoA-I, and subsequently cleaved within the cell to pro-apoA-I. Pro-apoA-I is then secreted into the circulation where the pro-segment is removed [13, 14]. Circulating apoA-I can interact with the ABCA1 transporter expressed on the surface of hepatocytes, lipid-loaded macrophages, and other peripheral cells. Upon apoA-I-ABCA1 interaction, cholesterol and phospholipids are transported from the donor cell to apoA-I, resulting in the formation of pre-β-HDL. These pre-β-HDL particles can again interact with ABCA1 on extrahepatic cells, enlarging the size of the HDL particles which as such contributes to reverse transport of cholesterol to the liver [15]. Therefore, increasing intestinal or hepatic secretion of apoA-I may be a promising strategy to reduce CHD risk. Quantification of plasma pro-apoA-I concentrations can be regarded as a measure of de novo apoA-I secretion [16]. It has been found that a high-fat meal increases apoA-I concentrations [8, 11], but if a high-fat meal stimulates de novo apoA-I secretion remains unclear. In addition, whether effects on postprandial apoA-I secretion differ between the three main macronutrients has not yet been studied. Therefore, the aim of this study was to compare effects of meals high in fat, protein, or carbohydrates on postprandial ABCA1-mediated CEC and apoA-I secretion in healthy overweight and slightly obese men.

Methods

Participants

Healthy overweight or slightly obese men were recruited from Maastricht and surrounding areas via advertisements in local newspapers, university and city buildings. In addition, men who had participated in earlier clinical studies and were willing to participate in other trials were approached. When interested, men received detailed information about the study and were invited for a screening visit. During this visit, participants first had to give their written informed consent. Then, weight and height were determined, and a fasted blood sample was obtained via venipuncture. Participants were included if the following criteria were met: aged between 18 and 70 years, BMI between 25 and 35 kg/m^2 with a stable body weight during the previous three months (<3 kg change), no medical condition or use of medication that might interfere with the study outcomes, no participation in other biomedical studies within 30 days before the start of and during the study, fasted total cholesterol concentrations < 8.0 mmol/L and triacylglycerol concentrations <2.2 mmol/L. The Medical Ethical Committee of Maastricht University and Medical Centre (MUMC+) had approved the protocol and the study was registered at ClinicalTrials.gov with identifier NCT03139890.

Study design

This double blind, randomized, crossover study consisted of three postprandial test days separated by a wash-out period of at least one week. During the test days, participants consumed a mixed meal high in either fats, carbohydrates, or proteins. Detailed information about the study design has been described previously [17]. Briefly, on each test day, participants came to the research unit by car or public transport after an overnight fast (from 8 PM). Participants refrained from alcohol the day before and did not exercise two days prior to the test day. On each test day, an intravenous cannula was inserted and a fasting blood sample (T0) was collected. Then, participants consumed one of the three meals and blood was sampled frequently for the next 4 hours. For this study, samples 60 min (T60) and 240 min (T240) after meal consumption were used, which were close to the maximal values of glucose, insulin and triacylglycerol [17]. The composition of the meals is shown in Table 1. Meals were isocaloric and each provided 953 kilocalories. The high-fat meal provided 52.3 % of energy (en%) from fats, 39.2 en% from carbohydrates, and 8.0 en% from proteins. For the high-carbohydrate meal these values were respectively 9.6 en%, 81.5, and 8.6 en%, and for the high-protein meal 10.6 en%, 51.5 en%, and 36.9 en%. Differences in volume between meals were corrected by providing additional glasses of water. Consequently, each meal had a volume of 730 mL.

Table 1. The compositions of the high-fat, high-carbohydrate and high-protein meals.

	High-fat	High-carb	High-protein
Energy, kcal	953	953	953
Fat, en% (g)	52.3 (55.3)	9.6 (10.2)	10.6 (11.3)
Saturated	31.3 (33.1)	3.2 (3.4)	3.8 (4.0)
Monounsaturated	15.1 (16.0)	3.8 (4.0)	3.8 (4.0)
Polyunsaturated	4.7 (5.0)	0.8 (0.9)	0.8 (0.9)
Carbohydrates, en% (g)	39.2 (93.5)	81.5 (194.3)	51.5 (122.7)
Protein, en% (g)	8.0 (19.2)	8.6 (20.4)	36.9 (87.9)
Water, g	297	262	115

Blood collection and biochemical analyses

Blood was sampled in serum separator vacutainer tubes (Becton, Dickinson and company, NJ, USA) for analyses of *ex vivo* CEC. Blood for analyses of pro-apoA-I and apoA-I concentrations was sampled in EDTA-plasma vacutainer tubes (Becton, Dickinson, and company). After sampling, serum tubes were allowed to clot for at least 30 minutes at room temperature and subsequently centrifuged at 1300×g for 15 minutes at 20°C. EDTA-plasma tubes were directly put on ice after sampling and immediately centrifuged at 1300×g for 15 minutes at 4°C. Serum and plasma aliquots were snap-frozen in liquid nitrogen and stored at -80°C until analysis.

All samples (T0, T60, and T240 from all three test days) from one subject were analyzed in the same analytical run. The *ex vivo* ABCA1-mediated CEC of HDL particles from lipid-loaded macrophages was quantified as previously described by de la Llera-Moya et al.[18] with minor modifications. Briefly, 50,000 J774 macrophages were seeded in 150 µL medium (DMEM low glucose; Lonza, Basel, Switserland) with 1% FBS (Biochrom, Cambridge, UK) and 1% antibiotics (Pen/Strep; Lonza) per well and incubated overnight with 2.5 µCi/ml ^{3}H-cholesterol (PerkinElmer, Waltham, MA, USA). After 4 hrs equilibration in 150 µL medium with 0.2% BSA (Sigma-Aldrich, St. Louis, MO, USA), ABCA1 was upregulated overnight via addition of 0.2 mM pCPT-cAMP (Sigma-Aldrich). During all assay steps, 1 µL/mL Acyl-CoA Acyltransferase (ACAT)-inhibitor (Sigma-Aldrich) was added to inhibit cholesterol esterification. Then, 2.8% apoB-depleted serum from a participant diluted in a mixture of PBS and medium plus 0.2% BSA was added in a total volume of 500 µL/well. A negative control without serum was included on each plate. After 4 hours of incubation, the amount of labeled cholesterol in the HDL fraction was quantified using liquid scintillation counting as a measure of CEC. To normalize CEC values, a standard serum pool with serum of healthy volunteers was included on each plate. The CEC value of this pool was set to 100% and participant values were expressed relative to the pool (% pool).

Plasma pro-apoA-I concentrations were determined using an ELISA as described by Gilham et al. [19]. Briefly, wells were coated overnight at 4°C with rabbit monoclonal anti-

proapoA-I antibody (kindly provided by Dr. Gilham and colleagues, Calgary, Canada). After blocking the wells for 1 hour using 5% skim milk and subsequent washing, plasma samples were added (20,000x diluted). C-terminal polyhistidine tagged recombinant proapoA-I (Genscript, Piscataway, NJ, USA) was used to prepare a calibration curve. After 90 minutes of incubation at room temperature and subsequent washing, mouse anti-human ApoA1 (Calbiochem, MercK KGaA, Darmstadt, Germany) was added and incubated for 60 minutes at room temperature (RT). Then, wells were washed and goat anti-mouse IgG-HRP antibody (Calbiochem, MercK KGaA, Darmstadt, Germany) was added and incubated for 40 minutes in the dark at RT. Lastly, wells were incubated with TMB in substrate buffer and absorbance was measured at 450nm after the enzymatic reaction was stopped by addition of H_2SO_4.

Plasma apoA-I concentrations were measured immunoturbidimetrically (Horiba ABX, Montpellier, France).

Statistical analyses

Baseline values at the start of the test days were compared using linear mixed models with subject as between subject variable and meal as fixed factor. Postprandial concentrations at T60 and T240 were compared to baseline concentrations for each meal separately using linear mixed models with subject as between subject variable and time as fixed factor. Differences in postprandial changes between the three meals were compared using linear mixed models with subjects as between subject variable, and period, meal, time, meal*time as fixed factors. If the interaction term reached statistical significance, time points were compared pairwise between meals with a Bonferroni post-hoc analysis. If the meal*time interaction term did not reach statistical significance, it was omitted from the model to assess the meal effect. Differences are reported as least squared means (LSM) with 95% confidence interval (CI) obtained from linear mixed models. Results were considered statistically significant when the p-value was < 0.05. Statistical analyses were performed using IBM SPSS Statistics for Mac, version 24.0 (IBM Corp., Armonk, NY, USA).

Results

Of the 23 participants screened, 20 were included and randomized. During the study period, two participants dropped out (Supplemental Figure 1). Baseline characteristics of the 18 men that completed the study are shown in Supplemental Table 1 and have already been published elsewhere [17]. Baseline ABCA1-mediated CEC and concentrations of apoA-I and pro-apoA-I at the start of each test day were comparable (p=0.212, p=0.604 and p=0.456, respectively; Table 2).

Cholesterol efflux capacity

Compared with baseline, ABCA1-mediated CEC did not change over time after intake of the high-fat (p=0.611), high-protein (p=0.122), or high-carbohydrate (p=0.908) meals (Table 2). Also, changes in ABCA1-mediated CEC over time did not dependent on the type of macronutrient intake (p=0.691 for meal*time-effect) and no differences between meals were found (p=0.443 for meal-effect; Supplemental Figure 2).

Apolipoprotein A-I

Compared with baseline, intake of the high-fat (time-effect: p=0.128), high-protein (p=0.070), or high-carbohydrate (p=0.456) meals did not significantly change apoA-I concentrations (Table 2). Postprandial changes over time for apoA-I were comparable between macronutrients (p=0.381 for meal*time-effect; Supplemental Figure 3). Also no meal-effect was observed (p=0.612).

Pro-apolipoprotein A-I

Compared with baseline, no significant changes were evident 60 minutes after each of the meals. After 240 minutes, however, pro-apoA-I concentrations were increased by 4.21 mg/L (95% CI: 0.25 to 8.18 mg/L; p=0.034) after the high-fat meal and by 10.4 mg/L (95% CI: 5.90 to 14.9 mg/L; p<0.001) after the high-protein meal, but not after the high-carbohydrate meal (3.83 mg/L; 95% CI: -0.48 to 8.14 mg/L; p=0.090; Table 2). Postprandial changes of pro-apoA-I differed over time between the macronutrients (meal*time-effect: p=0.021; Supplemental Figure 4). Pairwise comparisons showed that 240 minutes after meal intakes, increases in pro-apoA-I concentrations after the high-protein meal were 7.54 mg/L higher than after the high-carbohydrate meal (95% CI: 2.51 to 12.6 mg/L; p=0.001) and 5.78 mg/L higher than after the high-fat meal (95% CI: 0.79 to 10.8 mg/L; p=0.018). Changes 240 minutes after intake of the high-fat and high-carbohydrate meals did not differ.

Table 2. Postprandial ABCA1-mediated cholesterol efflux capacity, apolipoprotein A-I and pro-apolipoprotein A-I after intake of high-fat, high-carbohydrate, or high-protein meals.

	Time (min)	High-fat meal	High-carb meal	High-protein meal
CEC (% pool)	T=0	74.3 ± 9.7	75.5 ± 9.6	72.0 ± 12.1
	T=60	75.1 ± 7.8	75.47 ± 8.3	74.9 ± 12.4
	T=240	74.2 ± 8.8	74.9 ± 10.4	72.9 ± 8.4
ApoA-I (g/L)	T=0	1.24 ± 0.19	1.23 ± 0.18	1.24 ± 0.18
	T=60	1.26 ± 0.19	1.24 ± 0.19	1.26 ± 0.16
	T=240	1.25 ± 0.19	1.24 ± 0.19	1.24 ± 0.17
Pro-apoA-I (mg/L)	T=0	59.0 ± 22.3	56.4 ± 17.9	58.3 ± 16.9
	T=60	59.8 ± 21.2	58.3 ± 22.2	59.1 ± 18.4
	T=240	63.2 ± 23.4[a*]	60.3 ± 18.6[a]	68.7 ± 21.6[b*]

Values are means ± SD.
[a,b]Values within the same row with a different subscript are significantly different (P<0.05 with Bonferroni adjustment).
[*]Significantly different compared with its corresponding baseline (T=0) concentration (p<0.01).

Discussion

In this randomized, crossover study with healthy overweight and slightly obese men, no effects of high-fat, high-protein, or high-carbohydrate meals on postprandial ABCA1-mediated CEC or apoA-I concentrations were observed. Pro-apoA-I concentrations were, however, increased more pronounced 4 hours after intake of a high-protein meal than after intake of the high-fat or high-carb meals.

Earlier studies have shown that CEC increases 2 to 10 hours after intake of a high-fat meal [5-11]. We do not have an obvious reason why we did not observe an increase in ABCA1-mediated CEC after the high-fat meal that provided 55 g of fat. This amount is comparable to that used in other studies [5, 10, 11]. It can be hypothesized that the fatty-acid composition of the meal is important. Indeed, it has been reported that particularly oleic acid-rich fats enhance postprandial CEC when comparing four fats with different ratios of saturated, mono-, and polyunsaturated fatty acids (SFA:MUFA:PUFA) [5]. In our study, the main source of fat was butter, which is rich in saturated fatty acids (mainly palmitic acid (C16:0)). However, butter also contains oleic acid and the shake provided a total of 16 grams MUFA. Even though most other studies used fats that contained more MUFA and/or PUFA [5-10], the high-fat shake used by Talbot et al. had a fatty-acid composition comparable to ours (36 g SFA, 18.6 g MUFA, 4.1 g PUFA) [11]. Thus, differences in fatty-acid compositions are not a likely explanation. Another explanation may be that the methods used to measure CEC differed between studies. However, two studies used a method similar to ours, i.e. ABCA1-mediated CEC of apoB-depleted serum to pCPT-cAMP treated J774 macrophages [10, 11], and reported enhanced cholesterol efflux 2 [11] and 4 [10] hours after meal intake. Others measured cholesterol efflux mediated by SR-B1 instead of ABCA1 [5, 7, 9] and/or used whole

plasma or serum instead of solely the HDL fraction [5-9]. As we measured ABCA1-mediated CEC, we cannot exclude that other pathways involved in cholesterol efflux were changed after the high-fat meal. CEC did also not change after the high-protein and high-carbohydrate meals, suggesting that changes in postprandial glucose and/or insulin are not a major determinant of CEC. However, postprandial effects of meals rich in protein and carbohydrates on CEC have not been studied before and findings needs to be confirmed or refuted by other studies.

The different macronutrients may have different effects on fasting apoA-I concentrations [20]. Dietary fatty acids are one of the many factors known to influence apoA-I expression [21]. However, postprandial studies examining the effect of a high-fat meal on apoA-I concentrations are inconsistent, i.e. some studies did not report any differences between fasting and postprandial apoA-I concentrations [7, 22, 23], while others observed slight postprandial increases [8, 11]. In the present study, no differences in apoA-I concentrations after intake of the high-fat meal were observed. The number of studies examining the postprandial effects of the other macronutrients is scarce. However, Khoury et al. also observed no differences in postprandial apoA-I concentrations between high-fat, high-protein, or high-carbohydrate meals in subjects with or without the metabolic syndrome [23]. Also, Smolders et al. found in healthy overweight or obese men and women no differences in postprandial apoA-I concentrations between high-fat and high-carbohydrate meals [24]. Even though no differences were observed in plasma apoA-I, pro-apoA-I concentrations were increased 4 hours after intake of the high-fat or high-protein meals. Postprandial effects of macronutrients on pro-apoA-I have not been studied before, but two studies have measured the effects of a high-fat meal on pre-β-HDL. In one study, pre-β-HDL concentrations increased in overweight/obese women after a high-fat meal [9], while this was not observed in normal-weight women [9] and type IIB hyperlipidemic men [8]. Interestingly, effects on pro-apoA-I were most pronounced after the high-protein meal. We did not observe a direct relation between postprandial pro-apoA-I concentrations and apoA-I or ABCA1-mediated CEC. It is however possible that the pronounced effect of dietary protein on pro-apoA-I concentrations 4 hours after meal intake increases apoA-I and enhances ABCA1-mediated CEC at a later postprandial stage than measured here.

An intriguing question is how to explain the acute postprandial effects of the protein meal on pro-apoA-I concentrations observed 4 hours after meal consumption. The production of apoA-I is at least partly regulated via proliferator-activated receptor alpha (PPARα) [25, 26]. Dietary fatty acids are known ligands for PPARα, with MUFAs and PUFAs having a higher affinity than SFAs [27, 28]. It has even been suggested that higher concentrations of palmitic acid repress PPARα activation [29]. This may at least partly explain why pro-apoA-I concentrations increased only moderately after the high-fat meal and did not differ significantly from those after the high-carbohydrate meal. Whether dietary protein or specific amino acids increase pro-apoA-I via activation of PPARα or other regulatory

factors involved in apoA-I synthesis is not known. It is also possible that dietary protein indirectly affects apoA-I secretion, for example via effects of insulin. In an *in vitro* study, insulin induced apoA-I mRNA levels in HepG2 cells, while glucose inhibited mRNA levels [30]. This may provide an explanation for the higher pro-apoA-I concentrations after protein intake compared with carbohydrate intake, as protein intake only increased insulin while carbohydrate intake also increased glucose concentrations [17].

In conclusion, the intake of high-fat, high-protein, or high-carbohydrate meals did not affect postprandial ABCA1-mediated CEC or apoA-I concentrations, even though pro-apoA-I concentrations were increased after the high-protein and high-fat meals.

Acknowledgments

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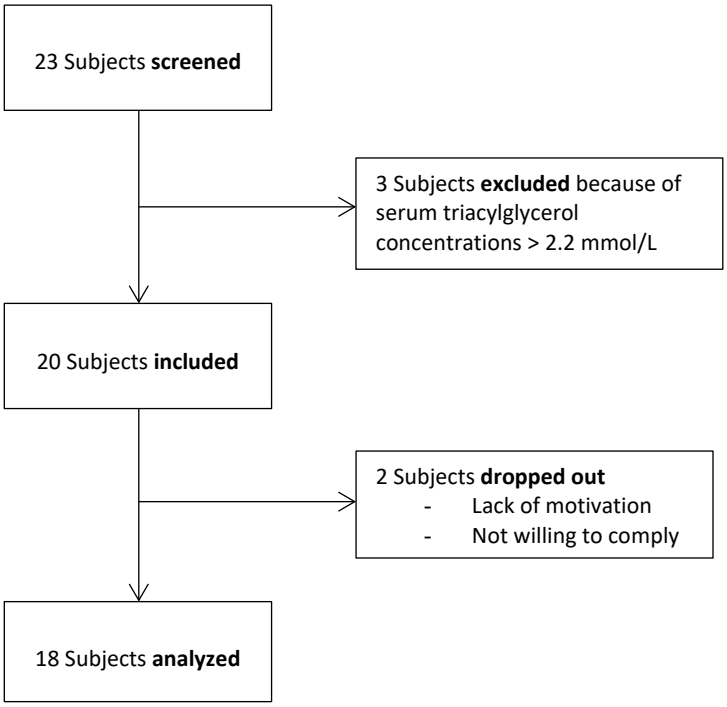
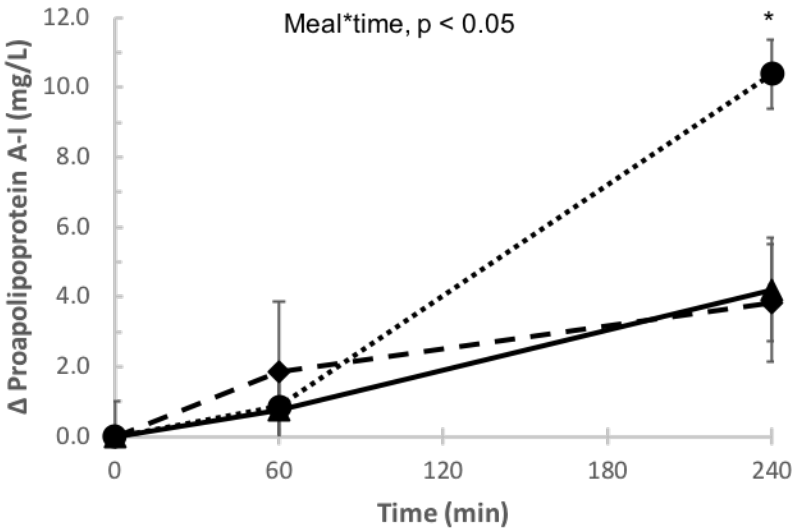
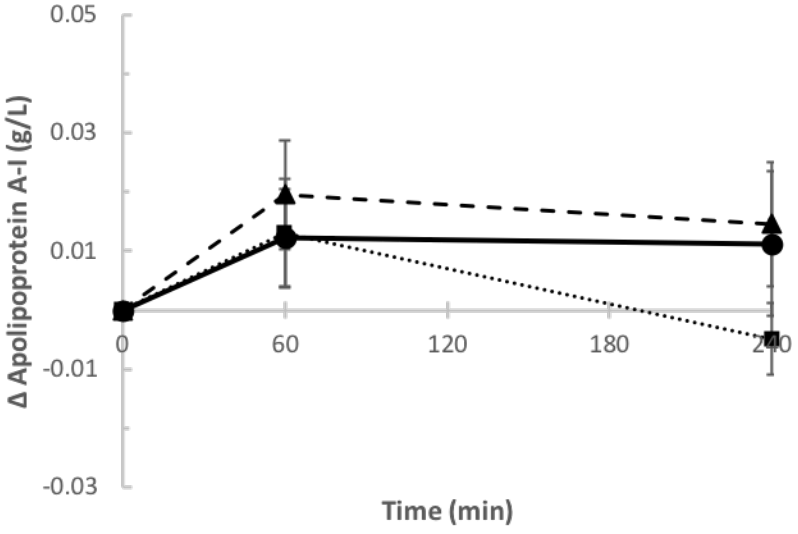
Supplemental Figures and Tables



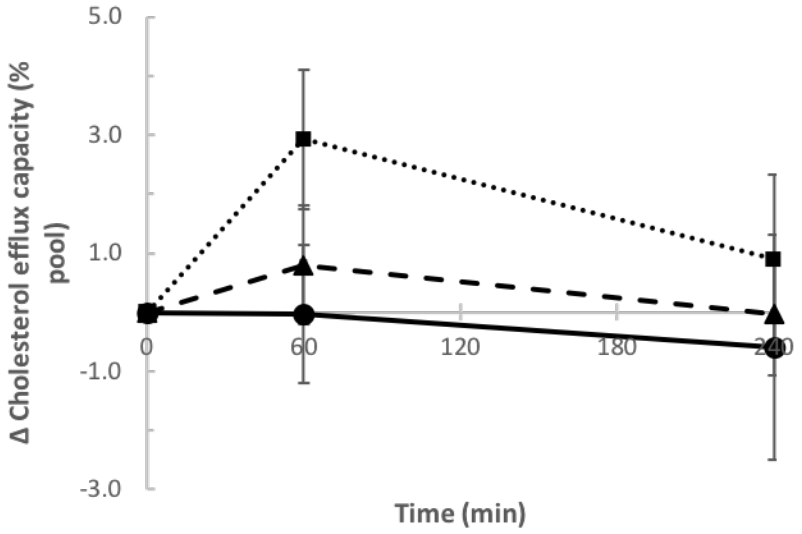
Supplemental Figure 1. Flowchart of the subjects screened, included and analyzed in the study.



Supplemental Figure 2. Postprandial time curves of proapolipoprotein A-I concentrations after intake of either the high-fat (▲), high-carbohydrate (●), or high-protein (■) meals. Data are presented as mean changes ± SEM (n=18) and were analyzed using linear mixed models. *Concentrations at 240 minutes were higher after the high-protein meal than after the high-carbohydrate (p=0.001) and high-fat (p=0.018) meals.



Supplemental Figure 3. Postprandial time curves of Apolipoprotein A-I concentrations after intake of either the high-fat (▲), high-carbohydrate (●), or high-protein (■) meals. Data are presented as mean changes ± SEM (n=18) and were analyzed using linear mixed models.



Supplemental Figure 4. Postprandial time curves of Cholesterol Efflux Capacity (CEC; expressed as % compared with the pool) after intake of either the high-fat (▲), high-carbohydrate (●), or high-protein (■) meals. Data are presented as mean changes ± SEM (n=18) and were analyzed using linear mixed models.

Supplemental Table 1. Baseline characteristics of the overweight and slightly obese men who completed the study. Values are reported as means ± SD unless otherwise noted.

Variables	Study participants (n=18)
Age (years)	65 (50.8 – 67.0)[a]
BMI (kg/m^2)	30.5 ± 2.9
Fasting serum total cholesterol (mmol/L)	5.32 ± 0.97
Fasting serum triacylglycerol (mmol/L)	1.27 ± 0.47
Fasting plasma glucose (mmol/L)	5.67 ± 0.49

[a] Median (IQR).

CHAPTER 7

General discussion

The social and economic burdens of cardiovascular diseases (CVD) such as coronary heart disease (CHD) are high and the number of CVD-related deaths is still growing [1]. Lifestyle, and more specifically diet, affects the risk of developing CHD. Therefore, nutritional interventions are important to target cardiometabolic health. Currently, dietary recommendations to prevent the development for CHD include guidelines to keep the intake of saturated fats below 10% of daily energy, for example by replacing products high in saturated fatty acids by products high in unsaturated fatty acids. However, saturated fat is an umbrella term for different saturated fatty acids that may exert different effects on serum lipids and other biomarkers of CHD. Furthermore, dietary guidelines are mainly based on the effects on fasted LDL-cholesterol, while evidence for associations between CHD-risk and other fasting biomarkers as well as for postprandial metabolism is growing. In addition, the causal association between HDL-cholesterol and CHD has been debated, as recent studies have suggested that we should not focus on HDL-cholesterol, but on HDL-functionality. HDL particles have multiple functionalities, but the ability to perform cholesterol efflux (cholesterol efflux capacity; CEC) is crucial for reverse cholesterol transport and has been negatively associated to atherosclerosis [2]. Therefore, the studies described in this dissertation focused on the effects of dietary fat and in particular of palmitic acid and stearic acid on both fasting as well as postprandial cardiometabolic risk markers including HDL-mediated cholesterol efflux. We have focused on palmitic acid and stearic acid because they are the two most abundant saturated fatty acids in Western diets. In addition, palmitic acid and stearic acid are often part of interesterified fats used by the food industry to improve characteristics of certain foods. The increasing use of interesterified fats may cause an exchange of dietary palmitic acid for stearic acid or vice versa.

Saturated fat and CHD risk

Already in 1977, the Select Committee on Nutrition and Humans Needs in the United Sates advised to keep the intake of saturated fats below 10% of daily energy [3]. Currently, this level of intake is still advised by the World Health Organization [4] and several countries such as the UK [5], US [6], and the Nordic countries [7]. In the Netherlands, dietary recommendations are based on food patterns rather than on nutrient intakes, but in general it is advised by the Netherlands Nutrition Centre (Stichting Voedingscentrum Nederland) to limit the intake of products rich in saturated fats [HCN 2015]. The guidelines for saturated fats are based on the effects of saturated fatty acids on LDL-cholesterol, as LDL-cholesterol is a well-known risk factor for CHD. Each 1 mmol/L reduction in LDL-cholesterol has been associated with a 21% decrease in CHD risk [8]. Many well-controlled randomized controlled intervention studies in various population groups have shown that LDL-cholesterol concentrations are higher on diets rich in saturated fats compared with diets rich in unsaturated fats [9]. However, three meta-analyses based on 21 [10], 20 [11], and 12 [12] cohort studies published in the last decade did not support the hypothesis that dietary saturated fatty acids are associated with CHD or

CVD risk. These findings should however be considered with caution as epidemiological studies also failed to find a relationship between saturated fat intakes and LDL-cholesterol [13]. Thus, it can be questioned if it is possible to show a relationship between saturated-fat intake and CHD risk at all. The lack of association between saturated-fat intake and LDL-cholesterol concentrations or CHD risk in these epidemiological studies can be related to several factors. First of all, it is challenging to reliably estimate food intake of an individual. For saturated fat, twenty-two randomly collected 24-h dietary recalls are needed to estimate the individual mean intake accurately [14]. Many epidemiological studies have only one food recall or a food frequency measure. Besides, many studies have not taken changes in food intakes over the years into account. Second, it is important to consider the macronutrient that replaces saturated fat. It has been shown that replacement of saturated fats with unsaturated fats and/or high-quality carbohydrates – but not with refined starches or added sugars – was associated with reduced CHD risk in two large cohorts [15]. Third, differences in saturated fat intake within a population are generally small, making it even harder to find associations. Fourth, endpoints differed between studies, as CVD risk was based on mortality, stroke, CHD, total CHD, fatal CHD, or incident coronary outcomes, while follow-up time also differed. LDL-cholesterol is mainly related to CHD. Fifth, even though lifestyle explains some of the variation in LDL-cholesterol concentrations between individuals, a major determinant of LDL-cholesterol concentrations is genetic background [16]. In fact, diet effects on LDL-cholesterol are important, but relatively small as compared with the absolute LDL-cholesterol concentration. Finally, conclusions were largely based on total saturated fatty acid intake, while the various saturated fatty acids might exert different metabolic effects. Recently, data from the Rotterdam study has been published in which a significant association was found between palmitic acid and CHD risk, but not for other saturated fatty acids or for total saturated fatty acid intake [17]. Indeed, well-controlled intervention studies have shown that individual saturated fatty acids have different effects on lipid metabolism. Stearic acid (C18:0), for example, lowers concentrations of LDL-cholesterol compared with palmitic acid (C16:0) [18, 19]. Taken together, there can be several reasons to explain that an association between saturated-fat intake and CHD risk is frequently not observed in epidemiological studies. Nevertheless, epidemiology provides the opportunity to study CHD-endpoints, while this is difficult to perform in dietary intervention studies.

HDL-mediated cholesterol efflux and CHD

High levels of HDL-cholesterol have been associated with a lower risk to develop CHD [20], but simply raising HDL-cholesterol concentrations with certain drugs failed to reduce cardiovascular events [21-23]. Therefore, it is now generally believed that we should focus on the functionality of HDL particles instead of HDL-cholesterol concentrations. In this dissertation, we have measured cholesterol efflux capacity (CEC) of HDL as a measure of HDL functionality because this is the first step of reverse cholesterol transport. As ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux is the predominant pathway of cholesterol efflux from lipid-loaded macrophages in the arterial wall to HDL particles [24] and has been inversely associated with atherosclerosis [2] and cardiovascular events [25, 26], we deliberately focused on this pathway in our studies. HDL constitutes of a heterogeneous group of particles and the smaller subfractions pre-β-HDL and HDL_3 are the most efficient ligands for cholesterol efflux mediated via ABCA1 [27]. In this dissertation, effects of palmitic acid and stearic acid on fasting (**chapter 3**) and postprandial (**chapter 5**) ABCA1-mediated CEC have been studied. In addition, we have examined the effects of the different macronutrients (fat, protein and carbohydrates) on postprandial ABCA1-mediated CEC (**chapter 6**). To quantify the ABCA1-mediated CEC of HDL particles *ex vivo*, you need 1) a cholesterol donor that mediates cholesterol efflux predominantly via ABCA1; 2) labeled cholesterol; and 3) a cholesterol accepter (**Figure 1**). Different donor cells can be used (e.g. tissue specific or isolated from blood, and from human or animal origin), but the contribution of the different transporters to mediate cholesterol efflux (ABCA1, ABCG1, SR-B1) as well as aqueous cholesterol diffusion depends on the cell system. We have used murine J774 macrophages incubated with pCPT-cAMP to upregulate ABCA1 expression. It is important to note that – although ABCA1 is the major contributor of cholesterol efflux in this cell system – also ABCG1-mediated cholesterol efflux and aqueous diffusion of cholesterol contribute to CEC [24, 28]. J774 macrophages were also treated with an Acyl-CoA Acyltransferase (ACAT)-inhibitor to prevent cholesterol esterification. As a tracer, different labels can be used. In **chapters 3 and 5** fluorescent BODIPY-labeled cholesterol was used, while in **chapter 6** radioactive ^{3}H-labeled cholesterol was used. Unpublished experiments from our group showed that efflux rates of both cholesterol labels are highly correlated. In addition, efflux rates as measured with either BODIPY-labeled cholesterol [29] or ^{3}H-labeled cholesterol [2] have been associated with cardiovascular events. Lastly, several cholesterol acceptors can be used depending on the pathway of interest. As we were interested in cholesterol efflux mediated by HDL, serum was depleted from apoB-containing lipoproteins. Besides the HDL fraction, apoB-depleted serum also contains small amounts of other cholesterol acceptors such as albumin and plasminogen [30]. In the end, the amount of cholesterol tracer present in the supernatant (apoB-depleted serum) after 4 hours of incubation was quantified as a measure of CEC. Because researchers often use different cholesterol efflux assays (e.g. different donors,

acceptors, labeled cholesterol, and incubation times), it may be difficult to compare results of different studies.

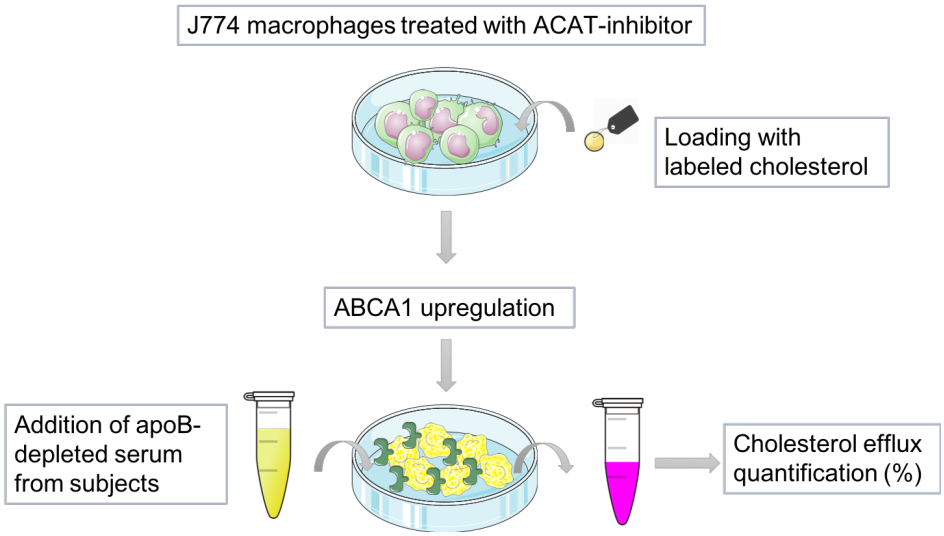


Figure 1. Schematic overview of the used cholesterol efflux capacity (CEC) assay. Abbreviations: ABCA1, ATP-binding cassette transporter A1; ACAT, Acyl-CoA Acyltransferase; apo, apolipoprotein.

Although CEC is negatively associated with the risk of developing CHD, it only provides information about one of the many steps involved in reverse cholesterol transport and does not mimic the dynamic *in vivo* situation. First of all, the ability of macrophages to export their excess cholesterol can be impaired. Indeed, it is known that macrophage function can be altered in metabolic disturbed conditions such as diabetes mellitus type II [31]. Macrophage dysfunction, however, is not accounted for in these cholesterol efflux assays. Second, the movement of cholesterol between a donor and acceptor can be bidirectional, while these assays only quantify a net unidirectional flux from the donor to the acceptor particles. Third, effective removal of extra-hepatic cholesterol also depends on among others LCAT activity for cholesterol esterification and SR-B1-mediated uptake of cholesterol by the liver. Fourth, cholesterol can also be exchanged between lipoproteins. Lastly, increasing evidence shows that not only the liver but also the small intestine plays a role in the excretion of cholesterol from the body, a pathway called transintestinal cholesterol efflux (TICE) [32]. Thus, taken together, it is not possible to directly translate results of CEC measurements to the complex pathways of cholesterol excretion in the human body.

Postprandial metabolism and CHD

Cardiometabolic risk markers are mainly measured during fasted conditions. However, people often spend most of the day in a postprandial state (approximately 18 hours), consuming three to five meals a day. There is increasing evidence that the way you coop with meal challenges is associated with the risk of developing metabolic disturbances and consequently atherosclerosis. Indeed, an impaired metabolism of postprandial triacylglycerol-rich lipoproteins (TRLs) contributes to the pathogenesis of CHD [33, 34] and is recognized as an independent risk factor for CHD [35, 36]. In healthy people, postprandial triacylglycerol concentrations peak 3 to 4 hours after meal-intake and have returned to baseline concentrations after 6 to 8 hours. When postprandial metabolism is impaired, postprandial triacylglycerol concentrations can be two to three times higher and can remain elevated up to 10 or even 12 hours after meal intake [34]. During the postprandial state, triacylglycerols present in TRLs (predominantly chylomicrons) are hydrolyzed by LPL to donate fatty acids mainly to adipose tissue. Due to triacylglycerol depletion, TRL-remnants are formed, which are smaller chylomicron and VLDL particles that are relatively rich in cholesteryl esters. Under normal conditions, TRL remnants are efficiently cleared by the liver. However, when this clearance is impaired, increased levels of remnants are present. Because TRL remnants are smaller, they can penetrate the arterial wall, causing lipid deposits, endothelial dysfunction, and inflammation, all hallmarks of atherosclerosis [37, 38]. Not only postprandial hypertriglyceridemia, but also postprandial hyperglycemia appears to be a better risk marker for CVD-related deaths than fasting glucose, also in subject without diabetes [39]. Hyperglycemia can cause endothelial dysfunction, as shown by a negative association between postprandial glucose concentrations and flow-mediated dilation (FMD) [40]. Examining postprandial metabolism is therefore of added value to study effects of diet on CHD risk. However, postprandial metabolism is very complex and the intake of dietary fat not only affects lipoprotein metabolism but also oxidative stress, inflammation and endothelial dysfunction [41]. In addition, studies that have examined the effects of macronutrients on postprandial metabolism are difficult to compare, because of differences in study designs such as meal composition, time between blood sampling, and postprandial follow-up. For studies examining effects of dietary fat, also the amount and type of fat may determine the postprandial response. In this dissertation, effects of palmitic acid and stearic acid on postprandial lipemia and glycemia have been described in chapter 4.

Second-meal effect

To examine postprandial metabolism, studies mainly used a single meal challenge, but to mimic the daily-life situation as closely as possible, the effects of multiple, consecutive meals should be studied. To implement this in clinical research settings is however not easy as participants need to be monitored for an even longer period of time, which is not only time consuming but also burdensome for the participants as well as the research team.

Performing a postprandial test that includes a second-meal challenge in a clinical research setting is therefore already a step forward. It is now recognized that upon meal-intake, chylomicrons are secreted that contain fatty acids from the previous meal that were stored within an enterocyte lipid pool [42-44]. This raises the question whether effects of specific fatty acids are more pronounced after intake of a second meal. In chapters 4 and 5, the results of a postprandial study in which we included a second-meal challenge are described. Lipid responses after the second meal differed from those after the first meal, i.e. the peak concentrations of triacylglycerols were reached earlier after the second meal, which agrees with previous studies [42, 45, 46]. Responses in parameters related to glucose and insulin metabolism as well as non-esterified fatty acids (NEFA) concentrations also differed between the first and second meals. Glucose, insulin, and C-peptide concentrations remained elevated for a longer time period after intake of the second meal. In addition, the differences between palmitic acid and stearic acid in postprandial NEFA concentrations and to a lesser extent in glucose and insulin markers were more evident after intake of the second meals. Thus, including a second-meal challenge may provide better insights in postprandial effects of dietary fatty acids.

Effects of palmitic acid and stearic acid on cardiometabolic risk markers

The main focus of this dissertation was to compare effects of palmitic acid and stearic acid on cardiometabolic risk markers. Palmitic acid and stearic acid are the two most abundant saturated fatty acids in Western diets. In addition, fats rich in palmitic and/or stearic acids are of interest for the food industry because they can be interesterified. Interesterification affects the physical properties of a fat including its melting behavior and thereby its suitability for the production of certain foods. Interesterified fats are nowadays widely used by the industry as they can replace partially hydrogenated *trans* fats, which are known to be more atherogenic. This increasing use of interesterified fats may affect intakes of palmitic and/or stearic acids. One main question is whether effects of palmitic acid and stearic acid on cardiometabolic health are different. The French Food Safety Agency (AFFSA) decided to exclude stearic acid from their dietary guidelines on atherogenic saturated fatty acids including lauric acid, myristic acid and palmitic acid [47]. Even though stearic acid lowers the CHD-risk factor LDL-cholesterol as compared with palmitic acid, it should be taken into account that CHD is a multifactorial disease. Thus, other factors are also involved in the progression of atherosclerosis thereby contributing to CHD risk. We have therefore studied effects of palmitic-acid versus stearic-acid intakes on lipids and (apo)lipoprotein concentrations, as well as HDL-mediated cholesterol efflux and markers related to glucose metabolism, low-grade inflammation and endothelial function. To get the most complete picture possible, we have measured most of these markers during the fasted state after a 4-week dietary intervention (longer-term) as well as postprandially.

Longer-term effects

In **chapter 3**, results of a dietary intervention study are described in which subjects consumed in a crossover design 4-week diets enriched with either palmitic acid or stearic acid. In line with earlier studies (as reviewed in **chapter 2**), the conventional risk marker LDL-cholesterol was lower when subjects consumed the stearic-acid diet compared with the palmitic-acid diet. The observed difference between the diets was 0.14 mmol/L. Assuming that a reduction of 1 mmol/L LDL-cholesterol is associated with a 21% decrease in CHD risk [8], this would suggest a decrease in CHD risk of approximately 3% on the stearic-acid diet. HDL-cholesterol and apoA-I concentrations were also lower on stearic acid, but ABCA1-mediated CEC was not. This suggests that the ability of the total HDL fraction to accept cholesterol from macrophages was not attenuated and that differences in CEC mediated via ABCA1 cannot explain the lower HDL-cholesterol concentration after stearic-acid intake. It is possible that lowering effect of stearic acid on HDL-cholesterol was at least partly due to the action of cholesteryl ester transfer protein (CETP), as CETP mass was higher on the stearic-acid diet. CETP is a protein that donates cholesterol from HDL particles to apoB-containing lipoproteins such as VLDL and LDL. Thus, an increased CETP activity can lower HDL-cholesterol concentrations. The decrease in apoA-I – the major protein on HDL particles – does not necessarily mean that there are less HDL particles, because HDL particles can contain two to four apoA-I molecules per particle. This is in contrast to apoB100 present on VLDL, IDL, and LDL particles, as each of these particles carries only one apoB100 molecule. In **chapter 5**, we have examined whether the decrease in apoA-I on the stearic-acid diet originates from a decrease in apoA-I secretion. However, no differences in apoA-I secretion were observed between the diets. Nevertheless, these results suggest that the capacity of pre-β-HDL and HDL_3 particles – the predominant acceptors of ABCA1-mediated cholesterol efflux [27] – was not negatively affected by the stearic-acid diet, even though HDL-cholesterol and apoA-I concentrations were lower.

To get more insight into the effects of palmitic-acid and stearic-acid diets on lipoprotein metabolism, we have also looked at their effects on cholesterol and triacylglycerol (TAG) concentrations within different VLDL, LDL, and HDL subfractions (**chapter 5**). Lipoproteins differ in size, density, and protein and lipid composition, and this determines their metabolic function. Small, dense LDL particles may for example be more atherogenic than larger, less dense LDL particles [48]. In contrast, small HDL particles are more efficient in facilitating cholesterol efflux via ABCA1 than larger HDL particles [30]. Our findings indicate that the lowering effect of stearic acid compared with palmitic acid on LDL-cholesterol occurred in all measured LDL subfractions, while the lower cholesterol concentrations in HDL particles were only present in large to medium HDL subfractions. The latter may explain why ABCA1-mediated cholesterol efflux was not different between diets. In contrast to cholesterol, TAG concentrations within some subfractions of VLDL, LDL, and HDL were increased on the stearic-acid diet. As TAG concentrations within lipoprotein

subfractions have been positively associated with myocardial infarction and ischemic stroke[49], this appears to be unfavorable.

Besides effects on lipoprotein metabolism, we have also examined other markers related to CHD (chapter 4). HOMA-IR, an accepted marker for insulin resistance, is positively associated with the risk of cardiovascular events [50]. We found that HOMA-IR was higher on the stearic-acid diet than on the palmitic-acid diet in postmenopausal women, but not in men. In contrast, Ng and colleagues did not observe sex differences when comparing effects of palmitic-acid and stearic-acid diets on insulin resistance, but this is may be due to the small number of men that was included [51]. Nevertheless, it is known that there are differences between men and women in fasting insulin resistance and that this probably is related to visceral adiposity, sex hormones, and adipokines. In general, females are more insulin sensitive than men, although this difference is diminished with menopause [52]. Whether postmenopausal women are indeed more sensitive to dietary changes in palmitic acid and stearic acid regarding insulin resistance needs to be confirmed by other studies. Our results also suggest that a stearic-acid diet unfavorably affects the proinflammatory markers IL-6 and TNF-α. The role of inflammation in the pathogenesis of atherosclerosis is well established [53]. In addition, IL-6 concentrations have been positively associated with HOMA-IR [54]. This is in line with our findings since the stearic-acid diet increased both inflammatory markers compared with palmitic acid as well as HOMA-IR (but the latter only in women). Nevertheless, although evidence is limited, other studies do not support the hypothesis that stearic-acid diets have adverse effects on insulin resistance [51, 55] or inflammation [55, 56] in (postmenopausal) women and men, although increased fibrinogen was observed after a stearic-acid diet [56].

Postprandial effects

To examine effects of palmitic-acid and stearic-acid intakes on postprandial lipemia and glycemia (chapter 4) as well as postprandial ABCA1-mediated CEC and apoA-I (chapter 5), a postprandial test was performed at the end of the 4-week diets with the same fatty acid as consumed during the preceding period (as described in chapter 4). During the postprandial tests, two consecutive mixed meals were provided that contained 50 grams of fat, 5 grams of protein, and 54 grams of carbohydrates. Almost half of the fatty acids in the meals were either palmitic acid or stearic acid. Stearic-acid intake lowered postprandial TAG concentrations as well as the number of chylomicrons compared with palmitic-acid intake. Other studies comparing the effects of these fatty acids on postprandial lipemia are controversial as reviewed in chapter 2. The discrepancy between studies may be explained by the different fat sources used for stearic acid (e.g. cocoa butter, shea butter, and allanblackia oil) as these differ in physical characteristics such as solid fat content at 37°C. Interesterification studies have shown that an increasing amount of solid fat at 37°C

attenuates postprandial lipemia [57]. Studies that observed a lower postprandial response after intake of the stearic acid-rich fats (including the study described in this dissertation) used stearic acid-rich fats that were not completely liquid at 37°C, while the palmitic acid-rich fats (mainly palm oil) were. Thus, this supports the hypothesis that the physical characteristics of the fat blends and not solely the amount of palmitic acid or stearic acid determines the magnitude of postprandial lipemia. However, to confirm this hypothesis, studies need to be performed where palmitic acid- and/or stearic acid-rich fats blend with different amounts of solid fat at 37°C are compared side-by-side. Nevertheless, the effects of the fat rich in stearic acid on postprandial lipemia appeared to be more favorable than the effects of the fat rich in palmitic acid.

The effects of both fatty acids on postprandial glycemia and insulin metabolism were less clear. No significant differences between palmitic-acid and stearic-acid intakes were observed in postprandial responses (iAUCs) of glucose, insulin, and C-peptide (a marker of insulin secretion), although there are indications that changes over time did depend on the fatty acid consumed. However, there were pronounced differences in the suppression of NEFA between the palmitic acid and stearic acid-rich meals, in particular after consumption of the second meals. During the entire 8-hour postprandial follow up, NEFA concentrations were lower after intake of the stearic-acid meals. In addition, the suppression of NEFA after the second palmitic-acid meal was delayed, while NEFA suppression occurred immediately after the second stearic-acid meal. The underlying mechanism of this phenomenon is unclear. It can be speculated that this may be a consequence of impaired adipocyte-insulin sensitivity after palmitic-acid intake, resulting in less suppression of adipocyte TAG-hydrolysis and/or less NEFA uptake [58]. Besides, differences between palmitic acid and stearic acid in postprandial NEFA concentrations may be related to the lower amount of circulating TAG after the stearic-acid meals, as fatty acid spillover from TAG hydrolysis of TRLs is at least one of the contributors to the plasma NEFA pool [59]. Nevertheless, these results suggest a more favorable effect of the stearic-acid meals on postprandial NEFA concentrations, which may at least partly be related to insulin sensitivity.

In addition, effects of palmitic-acid and stearic-acid intakes on changes in postprandial ABCA1-mediated cholesterol efflux to the HDL fraction and postprandial apoA-I concentrations were studied (chapter 5), but no differences were found. Also the postprandial secretion of apoA-I as measured by pro-apoA-I concentrations after intakes of palmitic-acid or stearic-acid meals was comparable.

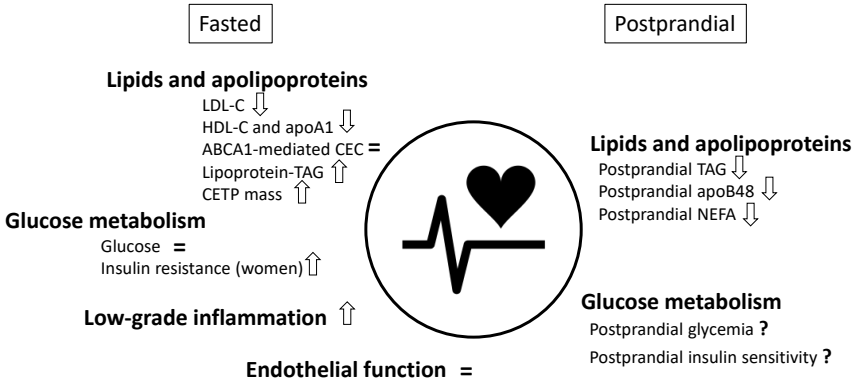


Figure 2. Schematic overview of the most important findings when substituting dietary palmitic acid with stearic acid on fasting and postprandial cardiometabolic risk markers. The arrows indicate the direction of the effect (higher or lower after stearic-acid intake versus palmitic-acid intake) and a '=' indicates that there was no difference between intakes.

Main conclusions and future recommendations

The findings of this dissertation have shown that the most commonly consumed saturated fatty acids palmitic acid and stearic acid differently affect several fasting and postprandial cardiometabolic risk markers involved in the pathogenesis of atherosclerosis. A simplified overview of the most important findings is given in Figure 2. The lowering effect of stearic acid on fasting LDL-cholesterol compared with palmitic acid is favorable. In addition, the observation that HDL-mediated cholesterol efflux via ABCA1 was not lowered on a stearic-acid diet, even though HDL-cholesterol and apoA-I concentrations were lower, suggests that this aspect of HDL functionality was not impaired. Besides, effects of stearic-acid intake on postprandial TAG and NEFA concentrations seem to be favorable, but these effects may be caused by the physical characteristics of the fats rather than the palmitic or stearic acid content. However, there appear to be less favorable effects of stearic-acid diets on fasting TAG concentrations within some lipoprotein subfractions, on fasting insulin sensitivity – although these effects seem to be sex-dependent – and on low-grade inflammation compared with palmitic-acid diets. Taken together, these findings do not exclude that palmitic acid and stearic acid differently affect cardiometabolic health via mechanisms other than LDL-cholesterol.

Currently, we are the first that have compared effects of dietary palmitic acid and stearic acid on the emerging risk marker HDL-mediated cholesterol efflux via ABCA1. Even though we did not observe significant differences, other efflux pathways such as cholesterol efflux via ABCG1 and SR-B1 should be studied as well to obtain a more detailed understanding on the effects of these two saturated fatty acids on total cholesterol efflux. However, the physiological importance of the ABCG1 and SR-B1 pathways is currently

unknown [60]. Also, mechanistic studies are warranted to explain how stearic acid and palmitic acid differently affect concentrations of LDL- and HDL-cholesterol. Furthermore, the observation that effects of palmitic-acid and stearic-acid diets on fasting insulin sensitivity are different between men and postmenopausal women needs to be confirmed. Although this has not been observed before, it has been found in other studies that effects of dietary fatty acids on CEC [61, 62] and postprandial TAG [63] are related to sex. Thus, men and women may respond differently to dietary interventions and more research in this field is needed. In addition, there may also be differences between pre- and postmenopausal women. Our findings have also suggested that conclusions after intake of consecutive meals may differ from those after a single meal challenge. Future studies should therefore consider including consecutive meals when examining postprandial responses. Ideally, continuous sensors should be used to monitor 24-hours fluctuations of biomarkers. We performed the meal challenges after subjects had consumed for 4-week diets rich in the corresponding fatty acid. To what extent the fatty-acid composition of the preceding diet influences postprandial response is unknown. Lastly, we have used blends of natural fats to study effects of palmitic acid and stearic acid. As there is increasing evidence that the solid fat content of stearic acid-rich fats at body temperature is an important determinant of postprandial lipemia [57], it needs to be examined whether the observed differences in postprandial responses are related to the fatty-acid composition (palmitic and/or stearic acid content) or the physical characteristics of the blends. For dietary recommendations, the question is how all these findings translate into long-term metabolic health. Therefore, future research should focus more on functional endpoints of cardiometabolic health such as FMD and pulse wave velocity (PWV).

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APPENDIX I

Summary
Samenvatting
Valorization

Summary

Coronary heart disease (CHD), also known as coronary artery disease (CAD) or ischemic heart disease (IHD), is a common type of cardiovascular disease (CVD) and a major cause of death worldwide. CHD is caused by a disrupted blood flow to the heart due to the development of an atherosclerotic plaque in the coronary arteries. LDL-cholesterol is a well-established risk factor for CHD, as high concentrations are positively and causally related to CHD. In contrast to LDL-cholesterol, high concentrations of HDL-cholesterol have been associated with a reduced risk for CHD. However, recent drug interventions that increased HDL-cholesterol failed to reduce this risk. Thus, HDL-cholesterol is not causally related to CHD and it is now believed that we should focus on HDL functionality instead of the concentration of HDL-cholesterol. One of the functionalities of HDL is its capacity to perform cholesterol efflux from lipid-loaded macrophages, known as cholesterol efflux capacity (CEC). Indeed, cholesterol efflux mediated via ATP-binding cassette transporter A1 (ABCA1-mediated CEC) has been inversely associated with CHD-risk. For ABCA1-mediated CEC, interaction between ABCA1 (present on many cells including macrophages) and apolipoprotein A-I (apoA-I; component of HDL) is crucial. Besides, also other fasted and postprandial markers are known to be relevant for the risk of developing CHD, such as those related to lipemia, glucose-insulin homeostasis, low-grade inflammation and/or endothelial function.

Diet and other lifestyle factors such as exercise or smoking affect the risk of developing atherosclerosis and subsequent CHD. One of the dietary factors that has been linked to CHD-risk is saturated fat, because the intake of saturated fat is positively associated with fasted serum LDL-cholesterol concentrations. However, saturated fat is an umbrella term for different saturated fatty acids that may exert different effects on LDL-cholesterol and other CHD-risk markers. Indeed, it is well-known that the two most abundant saturated fatty acids in many Western diets, palmitic acid (C16:0) and stearic acid (C18:0), differently affect cholesterol concentrations, i.e. stearic acid lowers LDL- and HDL-cholesterol compared with palmitic acid. However, it is less clear whether these fatty acids also differently affect other risk markers such as cholesterol efflux. Therefore, the research in this dissertation is mainly focused on the effects of dietary fat – predominantly the saturated fatty acids palmitic acid and stearic acid – on conventional and emerging cardiometabolic risk markers among which ABCA1-mediated CEC. For this, the results of a systematic review and two human dietary intervention studies have been described in this dissertation.

In chapter 2, existing literature describing the effects of stearic acid- versus palmitic acid-rich fats on cardiometabolic risk markers has been reviewed. In addition, it was also examined whether interesterification (shuffling fatty acids between and within triacylglycerols) of palmitic acid- or stearic acid-rich fats affects these risk markers, because interesterification is nowadays widely used by the food industry to increase the suitability of fats for certain

foods. Indeed, it was found that substituting palmitic acid with stearic acid lowers fasted serum LDL- and HDL-cholesterol. Interesterification of palmitic acid- or stearic acid-rich fats does not differently affect fasted lipids and (apo)lipoproteins when compared to their corresponding non-interesterified (native) fats. However, during the postprandial phase, lipemia is attenuated if the solid fat content of the fat blend at 37°C is increased by interesterification due to changes in palmitic acid or stearic acid *sn*-2 contents, while no evidence was found that solely substituting palmitic acid with stearic acid affects postprandial lipemia. How palmitic acid- or stearic acid-rich fats as well as interesterification of these fats affects other cardiometabolic risk markers needs further investigation before conclusions could be drawn.

In chapters 3, 4 and 5, the results of a human dietary intervention study with twenty men and fourteen postmenopausal women are described to examine longer-term and postprandial effects of palmitic-acid versus stearic-acid intakes on cholesterol efflux and other cardiometabolic risk markers.

The longer-term effects of 4-week diets rich in palmitic acids or stearic acids on cardiometabolic risk markers are reported in chapter 3. As expected, stearic-acid intake lowered fasted LDL- and HDL-cholesterol compared with palmitic-acid intake. ABCA1-mediated CEC was however comparable between the two diets, even though apoA-I concentrations were also lower on the stearic-acid diet. The lower HDL-cholesterol concentrations may at least partly be explained by an increased mass of cholesteryl ester transfer protein (CETP). In addition, the results suggest that insulin sensitivity in women as well as low-grade inflammation in both men and women might be unfavorably affected by stearic-acid intake.

In chapter 4, results of the postprandial tests performed at the end of both dietary intervention periods are described. Participants received two consecutive mixed meals high in palmitic acid- or stearic acid-rich fat blends during each postprandial test and effects on postprandial lipemia and glycemia were studied. Consumption of the meals containing the stearic acid-rich fat lowered postprandial lipemia as compared with the palmitic-acid meals. In addition, our results indicate that the number of chylomicrons after intake of the stearic-acid meals was lower. It is hypothesized that these observed differences are due to the higher solid fat content of the stearic acid-rich fat at 37°C. As triacylglycerol-rich lipoproteins are positively related to CHD, the effects of stearic acid on postprandial lipemia appear to be more favorable than those of palmitic acid. No pronounced differences in parameters related to postprandial glycemia were found between the fatty acids, although the changes over time in C-peptide – a marker of insulin secretion – differed. C-peptide concentrations were higher after the first stearic acid-rich meal and peaked earlier after the second stearic-acid rich meal when compared with the palmitic acid-rich meals. In addition, concentrations of non-esterified fatty acids (NEFA) were markedly lower after intake of the stearic acid-rich

fats. It is hypothesized that lower postprandial NEFA concentrations are more favorable, but a direct link between NEFA and CHD-risk has not been established so far.

Results of the 4-week diets on fasted lipoprotein subfractions, and fasted and postprandial apoA-I concentrations, apoA-I secretion and ABCA1-mediated CEC can be found in chapter 5. These results indicate that palmitic-acid and stearic-acid diets differently affect cholesterol and triacylglycerol concentrations in several VLDL, LDL, and HDL subfractions. Cholesterol concentrations on the stearic-acid diet were higher in subfractions of VLDL and lower in those of LDL and HDL compared with the palmitic-acid diet. On the other hand, triacylglycerol concentrations were higher in VLDL, LDL, and HDL subfractions on the stearic-acid diet. As triacylglycerol concentrations within lipoprotein subfractions have been positively associated with cardiovascular disease, this appears to be an unfavorable effect of stearic acid. No significant differences between the diets were found in fasted and postprandial ABCA1-mediated CEC as well as apoA-I secretion, although fasted apoA-I concentrations were lower on the stearic-acid diet.

Lastly, we have compared in eighteen men the effects of acute consumption of high-fat, high-protein, or high-carbohydrate meals on postprandial ABCA1-mediated CEC and (secretion of) apoA-I. These results are reported in chapter 6. We did not observe any differences between the meals in postprandial ABCA1-mediated CEC and apoA-I concentrations, even though the high-protein and high-fat meals increased apoA-I secretion.

In summary, the research in this dissertation was performed to get more insight into the effects of dietary fat, particularly the two most commonly consumed saturated fatty acids palmitic acid and stearic acid, on conventional and emerging cardiometabolic risk markers, with an emphasis on ABCA1-mediated CEC. Our findings have shown that palmitic acid and stearic acid have comparable effects on ABCA1-mediated CEC, but differently affect several other fasting and postprandial cardiometabolic risk markers. Thus, at this moment it cannot be excluded that palmitic acid and stearic acid differently affect cardiometabolic health via mechanisms other than LDL-cholesterol. In addition, no evidence was found that acute intakes of high-fat, high-protein or high-carbohydrate meals differently affect postprandial ABCA1-mediated CEC.

Nederlandse samenvatting

Coronaire hartziekten (CHZ), ook wel bekend als ischemische hartziekten, zijn de meest voorkomende hart- en vaatziekten en een veelvoorkomende oorzaak voor sterfte wereldwijd. CHZ worden veroorzaakt door een verminderde bloedtoevoer naar het hart vanwege aderverkalking (atherosclerose) in de coronaire slagaderen. Een welbekende risico factor voor CHZ is LDL-cholesterol, omdat hoge concentraties positief en causaal gerelateerd zijn aan CHZ. Naast LDL-cholesterol zijn er ook andere markers gerelateerd aan het risico op CHZ. Hoge concentraties HDL-cholesterol zijn bijvoorbeeld geassocieerd met een verlaagd risico op CHZ. Desalniettemin is het met recente farmacologische interventies die HDL-cholesterol verhogen niet gelukt om het CHZ-risico te verlagen. Daaruit is gebleken dat HDL-cholesterol niet causaal gerelateerd is aan CHZ en wetenschappers denken nu dat de functionaliteit van de HDL-deeltjes belangrijker is dan de hoeveelheid HDL-cholesterol. Een van de functies van HDL is het accepteren van cholesterol uit lipide-rijke macrofagen, dit noemen we cholesterol efflux capaciteit (CEC). Cholesterol efflux via ATP-bindende cassette transporter A1 (ABCA1-gemedieerde CEC) is negatief geassocieerd met het risico op CHZ. Voor ABCA1-gemedieerde CEC is interactie tussen ABCA1 (aanwezig op verschillende cellen waaronder macrofagen) en apolipoproteïne A-I (apoA-I; onderdeel van HDL) cruciaal. Daarnaast zijn ook andere nuchtere en postprandiale biomarkers relevant voor het risico op CHZ, zoals markers gerelateerd aan lipemie, glucose-insuline homeostase, lichte ontsteking en/of endotheel functie.

Dieet en overige leefstijlfactoren zoals sporten en roken hebben invloed op het risico om aderverkalking te ontwikkelen, wat weer kan leiden tot CHZ. Een van de factoren uit het dieet gelinkt aan het risico op CHZ is verzadigd vet, omdat de inname van verzadigd vet positief geassocieerd is met nuchtere serum LDL-cholesterol waarden. Verzadigd vet is echter een verzamelnaam voor verschillende verzadigde vetzuren die mogelijk verschillende effecten hebben op LDL-cholesterol en andere risicomarkers voor CHZ. Het is inderdaad bekend dat de twee meest voorkomende verzadigde vetzuren in menig Westers dieet, palmitinezuur (C16:0) en stearinezuur (C18:0), verschillende effecten hebben op het cholesterolgehalte. Stearinezuur verlaagd namelijk LDL- en HDL-cholesterol ten opzichte van palmitinezuur. We weten echter nog niet zo goed of deze vetzuren ook verschillende effecten hebben op andere risicomarkers zoals cholesterol efflux. Daarom ligt de focus van het onderzoek beschreven in dit proefschrift op de effecten van voedingsvet – voornamelijk de verzadigde vetzuren palmitinezuur en stearinezuur – op cardiometabole risicomarkers waaronder ABCA1-gemedieerde CEC. Hiervoor hebben we de resultaten van een systematische review en twee humane interventies studies beschreven.

In **hoofdstuk 2** is de bestaande literatuur over de effecten van vetten rijk aan stearinezuur versus vetten rijk aan palmitinezuur op cardiometabole risicomarkers besproken. Daarnaast is er ook gekeken of interesterificatie (uitwisselen van vetzuren tussen en binnen triglyceriden) van palmitine- of stearine-rijke vetten een effect heeft op deze risicomarkers, omdat interesterificatie momenteel veel wordt gebruikt door de voedingsindustrie om de toepasbaarheid van vetten te verhogen voor bepaalde voedingsmiddelen. Zoals verwacht verlaagd vervanging van palmitinezuur door stearinezuur de nuchtere serum concentraties van LDL- en HDL-cholesterol. Interesterificatie van vetten rijk in palmitinezuur of stearinezuur had geen effect op nuchtere lipiden en (apo)lipoproteïnen. Daarentegen was postprandiale lipemie verminderd als door interesterificatie de veranderingen in de hoeveelheid palmitine- of stearinezuur op *sn*-2 het gehalte aan vast vet bij 37°C verhoogden. Er was echter geen bewijs dat enkel het vervangen van palmitinezuur door stearinezuur een effect heeft op postprandiale lipemie. Er is meer onderzoek nodig om conclusies te trekken over de effecten van (interesterificatie van) vetten rijk aan stearinezuur of palmitinezuur op andere cardiometabole risicomarkers.

In **hoofdstuk 3, 4 en 5** zijn de resultaten beschreven van een humane voedingsinterventie studie met twintig mannen en veertien postmenopauzale vrouwen waarin zowel de langere-termijn als postprandiale effecten van de inname van palmitinezuur versus stearinezuur op cholesterol efflux en andere cardiometabole risicomarkers zijn bestudeerd.

De langere termijneffecten van een 4-weken dieet rijk in palmitinezuur of stearinezuur op cardiometabole risicomarkers zijn gerapporteerd in **hoofdstuk 3**. Zoals verwacht verlaagde de inname van stearinezuur nuchtere LDL- en HDL-cholesterol waarden vergeleken met de inname van palmitinezuur. Echter was er ondanks de verlaging van HDL-cholesterol en apoA-I-concentraties op het stearine-dieet geen verschil in ABCA1-gemedieerde CEC tussen de diëten. De lagere HDL-cholesterol concentraties kunnen mogelijk verklaard worden door de hogere hoeveelheid cholesterylestertransferproteïne (CETP). Daarentegen waren zowel insulinegevoeligheid in vrouwen als inflammatiewaarden in de gehele populatie mogelijk negatief beïnvloed door het stearine-dieet.

In **hoofdstuk 4** zijn de resultaten van de postprandiaal testen aan het eind van beide voedingsinterventies beschreven. Deelnemers kregen twee opeenvolgende maaltijden rijk aan palmitinezuur of stearinezuur tijdens elke postprandiaal test en de effecten op postprandiale lipemie en glycemie zijn bestudeerd. Inname van de maaltijden rijk aan stearinezuur verlaagde postprandiale lipemie vergeleken met de maaltijden rijk aan palmitinezuur. Daarbij laten de resultaten ook zien dat er minder chylomicronen waren na inname van de stearine-rijke maaltijden. De hypothese is dat deze verschillen veroorzaakt worden door het hogere gehalte aan vast vet in het stearine-rijke vet bij lichaamstemperatuur. Aangezien triglyceride-rijke lipoproteïnen positief gerelateerd zijn aan CHZ, lijken de effecten van stearinezuur op postprandiale lipemie gunstiger te zijn dan die

van palmitinezuur. Er waren geen uitgesproken verschillen waarneembaar in markers gerelateerd aan postprandiale glycemie, al waren de veranderingen over tijd in C-peptide – een marker voor insuline secretie – verschillend tussen de vetzuren. De concentratie C-peptide was hoger na de eerste stearine-rijke maaltijd en piekte eerder na de tweede stearine-rijke maaltijd ten opzichte van de palmitine-rijke maaltijden. Daarbij was de concentratie vrije vetzuren sterker verlaagd na inname van de stearine-rijke maaltijden. De hypothese is dat lagere postprandiale vrije vetzuren gunstiger zijn, maar een directe link tussen vrije vetzuren en het risico op CHZ is tot op heden niet vastgesteld.

Resultaten van de 4-weken diëten op nuchtere lipoproteïnen subfracties alsmede op nuchtere en postprandiale apoA-I waarden, apoA-I secretie en ABCA1-gemedieerde CEC zijn gerapporteerd in hoofdstuk 5. Deze resultaten laten zien dat diëten rijk aan palmitine of stearinezuur verschillende effecten hebben op nuchtere cholesterol en triglyceride concentraties in meerdere VLDL, LDL en HDL subfracties. Cholesterolwaarden tijdens het stearine-dieet waren hoger in subfracties van VLDL en lager in subfracties van LDL en HDL vergeleken met het palmitine-dieet. Daarentegen waren de triglyceride concentraties in VLDL, LDL en HDL subfracties hoger op het stearine-dieet. Aangezien triglyceride concentraties binnen lipoproteïne subfracties positief geassocieerd zijn met hart- en vaatziekten, lijkt dit een ongunstig effect van stearinezuur. Er zijn geen significante verschillen gevonden tussen diëten in nuchtere en postprandiale ABCA1-gemedieerde CEC alsmede apoA-I secretie, ondanks dat nuchtere apoA-I concentraties lager waren op het stearine-dieet.

Tot slot hebben we in achttien mannen de effecten vergeleken van de acute consumptie van hoog-vet, hoog-eiwit of hoog-koolhydraat maaltijden op postprandiale ABCA1-gemedieerde CEC en (secretie van) apoA-I. Deze resultaten zijn beschreven in hoofdstuk 6. Er zijn geen verschillen gevonden tussen de maaltijden wat betreft serum ABCA1-gemedieerde CEC en apoA-I concentraties, terwijl de hoog-vet en hoog-eiwit maaltijden apoA-I secretie verhoogden.

Het onderzoek in dit proefschrift was uitgevoerd om meer inzichten te krijgen in de effecten van voedingsvetten, voornamelijk de twee meest geconsumeerde verzadigde vetzuren palmitinezuur en stearinezuur, op een breed scala aan cardiometabole risicomarkers, met de nadruk op ABCA1-gemedieerde CEC. Onze bevindingen hebben laten zien dat palmitinezuur en stearinezuur vergelijkbare effecten hebben op ABCA1-gemedieerde CEC, maar verschillende effecten hebben op een verscheidenheid aan andere nuchtere en postprandiale cardiometabole risicomarkers. Op dit moment kan het dus niet uitgesloten worden dat palmitinezuur en stearinezuur verschillende effecten hebben op de cardiometabole gezondheid via mechanismes niet gerelateerd aan LDL-cholesterol.

Daarnaast laat het onderzoek in dit proefschrift zien dat er geen verschillen zijn tussen innames van hoog-vet, hoog-eiwit of hoog-koolhydraat maaltijden in postprandiale ABCA1-gemedieerde CEC.

Valorization

Societal and economic relevance

Over the past decades, cardiovascular diseases (CVDs) have been the leading cause of death worldwide [1]. Currently, the number of CVD-related deaths is still growing. In 2016, approximately one third of all global deaths was due to CVDs. In addition, living with CVD or having a high CVD-risk lowers the health-related quality of life including physical and mental health [2]. The high number of CVDs also results in a high economic burden. In 2010, the global costs of CVDs added up to approximately 863 billion US dollars and it has been estimated that these costs will increase to 1044 billion US dollars in 2030 [3]. In Europe alone, the costs of CVDs are estimated to be 210 billion euros per year [4].

Coronary heart disease (CHD) is a common type of CVD and is often caused by atherosclerosis. Diet is one of the underlying causes for developing CHD and therefore dietary recommendations are needed to prevent CHD [1]. In order to develop these recommendations, dietary intervention studies are required that unravel the complex relationship between nutrition and CHD-risk. It is well-known that dietary saturated fat increases LDL-cholesterol, a risk factor for CHD, compared with unsaturated fats. It is therefore advised to keep the intake of saturated fat below 10% of daily energy [5]. However, saturated fat is an umbrella term for different saturated fatty acids that may exert different effects on CHD-risk factors. In fact, stearic acid (C18:0) does not increase LDL-cholesterol compared with carbohydrates [6] and lowers concentrations of LDL-cholesterol compared with other saturated fatty acids such as palmitic acid (C16:0) [7]. As palmitic acid and stearic acid are the most abundant saturated fatty acids in many Western diets, it is important that their potential differences in metabolic effects are considered when developing dietary guidelines for saturated fat intake. Based on the effect of stearic acid on LDL-cholesterol, the French Food Safety Agency (AFSSA) decided to exclude stearic acid from their dietary guidelines on atherogenic saturated fatty acids [8]. However, as CHD is a multifactorial disease, LDL-cholesterol is not the only underlying factor that contributes to the development of CHD. Although the effects of stearic acid compared with palmitic acid on LDL-cholesterol are well-known, the effects on other risk markers have been studied less extensively. Thus, more research is needed to draw conclusions about their effects on CHD-risk beyond LDL-cholesterol and get a comprehensive overview of their impact on cardiometabolic health. Therefore, we have focused in this dissertation on the effects of the most commonly consumed saturated fatty acids palmitic acid and stearic acid on conventional and emerging cardiometabolic risk factors. Ultimately, the findings of this research combined with findings of other scientists may provide underlying evidence for the development or revision of dietary guidelines. These guidelines will help the society to improve diet quality and will eventually contribute to the prevention of CHD and to public health in general.

Commercial relevance

As it is generally advised to reduce the intake of dietary saturated fatty acids, it is of great interest to the food industry to replace animal fats that are generally rich in saturated fatty acids with vegetable oils and fats rich in unsaturated fatty acids. In fact, the food industry nowadays heavily relies on the use of vegetable oils and fats for the production of processed foods, for example margarines and baked goods. However, for these food products, certain physical characteristics of the fats are required such as a specific melting behavior or solid fat content. To increase the suitability of vegetable oils and fats for the food industry, modification processes are used. For a long time, partial hydrogenation of vegetable oils was widely used. However, besides the formation of saturated fatty acids, also *trans* fatty acids were formed with partial hydrogenation. These industrially produced *trans* fatty acids are nowadays not allowed anymore as they have unfavorable effects on serum lipids and thereby increase the risk of developing CHD. An alternative to partial hydrogenation is interesterification, also known as the randomization of fats. Interesterification is a modification process that rearranges the fatty acids between and within triacylglycerol molecules resulting in new triacylglycerol species that have different physical characteristics, but without changing the overall fatty acid composition of the fat. Thus, in contrast to partial hydrogenation, no *trans* fatty acids are formed by interesterification [9]. However, even though no trans fatty acids are formed, it has been speculated that the position of the fatty acids within the triacylglycerol molecule determines its metabolic faith. More specifically, fatty acids at the *sn*-2 position are believed to remain attached to the glycerol backbone and end up in the liver where they will subsequently be incorporated into lipoproteins that enter the circulation. It may therefore be possible that interesterification of fats also affects cardiometabolic health. Vegetable fats and oils used for interesterification are often rich in palmitic acids and/or stearic acids. Normally, palmitic and stearic acids are mainly present at the outer *sn*-1 and *sn*-3 positions, but with interesterification the amount of these fatty acids at *sn*-2 increases. Thus, it is important that it is well-studied whether this position within the triacylglycerol molecules affects the metabolic faith of palmitic and/or stearic acids and thereby cardiometabolic health. As the existing literature on the effects of interesterification of palmitic acid- or stearic acid-rich fats is described in this dissertation, these results are also very relevant for the food industry. In addition, because stearic acid has a lowering effect on serum LDL-cholesterol compared to other saturated fatty acids, it has even been suggested that stearic acid-rich fats may be interesting for the production of functional foods

Environmental relevance

In the research described in this dissertation, we have predominantly studied effects of palm oil (rich in palmitic acids) and allanblackia oil (rich in stearic acids) on cardiometabolic health. These oils are both suitable for the production of interesterified fats. Palm oil, derived from the fruits of oil palm trees mainly found in Asia (i.e. Indonesia and Malaysia), is the most

commonly consumed vegetable oil. Palm oil consumption accounts for 30% of oil consumption worldwide. Almost half of the fatty acids in palm oil are palmitic acids. It has been estimated that around 75 million tonnes of palm oil will be consumed in 2019-2020 [10]. Although the production of palm oil is very efficient (one oil palm can produce up to 4000 liters of oil) and a good income source for the local producers, many people are concerned about the use of palm oil as its increased cultivation has among others resulted in negative effects on plant and animal biodiversity of some tropical forests, particularly in Malaysia. A large number of companies and organizations therefore work together to increase sustainability of the production of palm oil (also known as the Round Table on Sustainable Palm Oil). In the Netherlands, 90% of the palm oil used nowadays is sustainable. Nevertheless, due to the increasing world population and consumer society, it is still important to also use other vegetable oils and fats when possible. Allanblackia oil is derived from the fruit seeds of the Allanblackia tree commonly found at tropical forests in Africa (i.e. Tanzania and Ghana). The physical characteristics (solid at room temperature) of allanblackia oil, that are mainly due to its high stearic acid content (more than half of its fatty acids), make this oil very suitable to use as a structuring fat by the food industry [11]. Currently, allanblackia seeds are mainly harvested in the wild, but this wild harvesting alone will not meet long-term demands. Therefore, the 'Allanblackia Partnership' has been founded to increase the use and production of allanblackia oil in a sustainable way.

Translation into practice

Given that palmitic acid and stearic acid are the most abundant saturated fatty acids in many Western diets and that fats rich in palmitic and/or stearic acid are often used for interesterification by the food industry, it is important that we thoroughly understand their impact on metabolic health. Of course, this research alone is not enough to reconsider dietary guidelines for saturated fat nor to advise the food industry on which fats to use. Nevertheless, the reported findings of palmitic-acid and stearic-acid intakes on a broad selection of conventional and emerging cardiometabolic risk markers provide a foundation for future studies to confirm or refute these results. The question remains how all these findings translate into long-term metabolic health. Therefore, future research should also focus on functional cardiometabolic endpoints. The findings described within this dissertation were presented at several (inter)national congresses. In addition, the findings will be published in scientific journals and thus be publicly available for scientists as well as for policy makers and the food industry.

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APPENDIX II

Dankwoord
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Dankwoord

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About the author

Merel Anne van Rooijen was born on March 17, 1992 in Breda, the Netherlands. She completed secondary school in 2010 at the Mencia de Mendoza Lyceum in Breda and moved to Wageningen to study Nutrition and Health at Wageningen University & Research. In 2013, she wrote her bachelor thesis at the department of Cell Biology and Immunology and received her Bachelor of Science degree. Merel continued with the master Nutrition and Health, specialization Molecular Nutrition and Toxicology. Her master thesis was performed at the department of Nutrition and Pharmacology of Wageningen University & Research. Thereafter, she continued with a 6-month internship at the Immunology department of Early Life Nutrition, Nutricia Research in Utrecht and received her Master of Science degree in 2015. Merel then moved to Maastricht to start her PhD project in March 2016 supervised by prof. dr R.P. Mensink and prof. dr J. Plat at the Physiology of Human Nutrition research group within the department of Nutrition and Movement Sciences at Maastricht University (Faculty of Health, Medicine and Life Sciences). During her PhD trajectory, she conducted a human dietary intervention trial to study the effects of the saturated fatty acids palmitic acid and stearic acid on cardiometabolic health. In addition, she was involved in teaching activities and obtained the University Teaching Qualification. In 2019, Merel was awarded with the Foppe ten Hoor Young Investigator Award for presenting her work at the Nutritional Science Days in Heeze. The research conducted during her PhD trajectory is described in this dissertation entitled 'Effects of dietary fat on cholesterol efflux and other cardiometabolic risk markers in humans - focus on palmitic acid and stearic acid -'.

List of publications

van Rooijen MA, Mensink, RP. Palmitic acid versus Stearic acid: Effects of interesterification and intakes on cardiometabolic risk markers - a systematic review. *Nutrients 2020, 12*, *615*.

van Rooijen MA, Plat J, Blom WAM, Zock PL, Mensink RP. Dietary palmitic acid and stearic acid do not differently affect ABCA1-mediated cholesterol efflux in healthy men and postmenopausal women: a randomized controlled trial. *Submitted*

van Rooijen MA, Plat J, Blom WAM, Zock PL, Mensink RP. Effects of two consecutive mixed meals high in palmitic acid or stearic acid on 8-hour postprandial lipemia and glycemia in healthy men and postmenopausal women. *To be submitted*

van Rooijen MA, Plat J, Jacobs DM, Blom WAM, Mensink RP. Effects of dietary palmitic acid and stearic acid on lipoprotein subfractions, ABCA1-mediated cholesterol efflux and apoA-I secretion in healthy men and postmenopausal women. *To be submitted*

van Rooijen MA, Plat J, Joris PJ, Smeets ETHC, Mensink RP. A comparison of the postprandial effects from high-fat, high-protein or high-carbohydrate meals on ABCA1-mediated cholesterol efflux and apoA-I secretion in overweight or slightly obese men. *To be submitted*