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DIFFERENTIAL EFFECTS OF SATURATED FATTY ACIDS OF VARYING CHAIN LENGTH ON LIPID PROFILES IN HEALTHY INDIVIDUALS.

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Statement of originality

I hereby certify that the work embodies in the thesis is my own work, conducted under normal supervision.

The thesis contains published scholarly work of which I am a co-author. For each such work a written statement, endorsed by the other authors, attesting to my contribution to the joint work has been included.

The thesis contains no material which has been accepted, or is being examined, for the the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give my consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provision of the Copyright Act 1968.

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Nisha

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Nisha Panth

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…..we know how to wait…. ….

…..let the stars keep on turning….. ….

…..from little things….big things grow…..

Paul Kelly

LIST OF RESEARCH PUBLICATIONS INCLUDED IN THE THESIS

1. **Chapter 3**

Panth N, Abbott KAA, Dias C, Wynne Katie, Garg ML. Differential effects of medium and long-chain saturated fatty acids on blood lipid profile: a systematic review and meta-analysis; The American Journal of Clinical Nutrition. 2018; 108(4): 675-687. DOI: 10.1093/ajcn/nqy167

2. **Chapter 4**

Panth N, Dias C, Singh H, Wynne Katie, Garg ML. Medium-chain fatty acids lower postprandial lipemia: a randomized crossover trial. 2018; submitted to Clinical Nutrition.

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ABBREVIATIONS

CVD	Cardiovascular disease
SFA	Saturated fatty acid
SCFA	Short-chain saturated fatty acid
MCFAS	Medium-chain saturated fatty acid
LCFA	Long-chain saturated fatty acid
apo A-I	Apolipoprotein A-I
TG	Triglyceride
TC	Total cholesterol
LA	Linoleic acid
AA	Arachidonic acid
PUFA	Polyunsaturated fatty acids
n-3PUFA	Omega-3 polyunsaturated fatty acids
n-6PUFA	Omega-6 polyunsaturated fatty acids
sdLDL	Small dense low-density lipoprotein
CLA	Conjugated linoleic acid
EPA	Eicosapentaenoic acid
DPA	Docosapentaenoic acids
DHA	Docosahexaenoic acid
TFA	Trans-fatty acids
NEFA	Non-esterified fatty acids
ACC	Acetyl-CoA carboxylase
FASN	Fatty-acid synthase
CoA	Coenzyme A
VLDL	Very low-density lipoprotein
IDL	Intermediate-density lipoprotein

MUFA	Monounsaturated fatty acids
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA
apoC-III	apolipoprotein C-III
apo B	Apolipoprotein B
apo A-IV	apolipoprotein A-IV
apoE	apolipoprotein E
apo B-100	Apolipoprotein B-100
apo B-48	Apolipoprotein B-48
FFA	Free fatty acid
LPL	Liporotein lipase
ABCA-1	ATP-binding cassette sub-family A member 1
CETP	Cholesteryl ester transfer protein
LCAT	Lecithin-cholesterol acyltransferase
SR-B1	Scavenger receptor class B type 1
TRL	Triglyceride rich lipoprotein
iAUC	Incremental area under the curve
AUC	Area under the curve
MCT	Medium chain triglyceride
PPARs	Peroxisome proliferator activated receptors
LXRs	Liver X receptors
HNF 4	Hepatic nuclear factor 4
SREBPs	Sterol regulatory element binding proteins
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
HDL-P	High density lipoprotein particles
HDL-TG	High density lipoprotein triglycerides

iAUC	Incremental area under the curve
IDL-C	Intermediary density lipoprotein cholesterol
IDL-TG	Intermediary density lipoprotein triglycerides
LCAT	Lecithin-cholesterol acyltransferase
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein cholesterol
LDL-P	Low density lipoprotein particle
LDLr	Low density lipoprotein receptor
LDL-TG	Low density lipoprotein triglycerides
MUFA	Monounsaturated fatty acids
NEFA	Non-esterified fatty acids
n-6 PUFA	Omega-6 polyunsaturated fatty acids
n-3 PUFA	Omega-3 polyunsaturated fatty acids
PPAR	Peroxisome proliferator-activated receptor
SREBP-1c	Sterol regulatory element-binding protein 1c
TAG	Triacylglycerol
VLDL	Very low-density lipoprotein
VLDL-C	Very low-density lipoprotein cholesterol
VLDL-P	Very low-density lipoprotein particle
VLDL-TG	Very low-density lipoprotein triglycerides
CHD	Coronary heart disease
HDL: LDL-C	HDL-to-LDL cholesterol ratio
LDL: HDL	LDL-to-HDL cholesterol ratio;
Lp(a)	Lipoprotein (a)
mRNA	Messenger RNA
RCT	Reverse cholesterol transport

TC: HDL	Total cholesterol-to-HDL ratio
CB	Coconut biscuit
BB	Butter biscuit
LB	Lard biscuit

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SYNOPSIS

Saturated fatty acid (SFA) intake has been linked with increased blood lipid concentrations and increased cardiovascular disease (CVD) risk. Current dietary guidelines consider all SFA as a single group and encourage to reduce saturated fat consumption to 10% of daily energy intake. However, not only the number, position and configuration of double bonds, but also the chain length of SFA have been shown to be a major determinant of their metabolic fate. Short-chain (SCFA, 2-4 carbons long, found in butter and products of fibre and resistant starch fermentation) and medium-chain (MCFAS, 6-12 carbons long, found in coconut and palm kernel oil) are absorbed directly through the villi of the intestinal mucosa and transported to the liver via the portal circulation. In contrast, long-chain (LCSFAS, >12 carbons, found in animal fats and dairy products) follow complex metabolic pathways including chylomicron synthesis in the intestinal villi, secretion into the thoracic lymph, hydrolysis of some triglycerides by lipoprotein lipase into free fatty acids which are transferred to the peripheral tissues (including muscle) followed by chylomicron remnants being taken up into the liver for further metabolism. This, together with the fact that humans spend most of their time in the postprandial state, suggests that saturated fat type may play an important role in overall lipid metabolism and modulation of CVD risk. Therefore, it was hypothesised that consumption of SCFA and MCFAS reduce blood lipid levels compared with LCSFAS.

Our first aim, addressed in chapter 3, was to to establish the basis for our hypothesis, conducting systematic review and meta analysis of the literature assessing the differential effects of chain lengths of SFA on blood lipids. The findings from this chapter demonstrate that the consumption of MCFAS enriched diets increased high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-I (apo A-I) levels compared to LCSFAS diet without any significant effect on triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels.

In chapter 4, we aimed to determine if SFA of different chain lengths would differentially influence postprandial lipid levels. In a randomised cross-over design, we investigated the effect of a meal (sweet biscuits) rich in either SCFA or MCFAS or LCSFAS on postprandial lipids (TG, TC, LDL-C, and HDL-C). The results presented in this chapter demonstrate that the postprandial triglyceride response following MCFAS

was lower compared to LCSFAS and that predominant fatty acid in the meal is a determinant of the lipemic response.

In conclusion, while this study has highlighted the differential effects of chain lengths of SFA on blood lipids. These results draw attention to the evidence that guidelines on SFA must consider the fatty acid chain length and importantly, the diverse SFA containing foods (processed and unprocessed meats, dairy products, eggs, coconut and palm oils, chocolate) that may possess harmful, neutral or even beneficial effects in relation to cardiovascular health.

THESIS LAYOUT

This thesis by publication is organised in 5 chapters, including one published study in the American Journal of Clinical Nutrition. This thesis contains a general introduction and literature review section (Chapter 1) followed by the methodology undertaken in the conduct of the research (Chapter 2). The introduction, methods, results, discussion of the research conducted for this thesis are then demonstrated as Chapter 3 and Chapter 4. This thesis presents work from a body of research comprised of one systematic review/ meta-analysis and one human research study; (i) differential effects of medium and long-chain saturated fatty acids on blood lipid profile: a systematic review and meta-analysis (Chapter 3) and (ii) medium chain fatty acids lower postprandial lipemia: a randomized, crossover trial (Chapter 4). An overall discussion of the findings from this body of research and its implications are demonstrated in the final chapter of the thesis (Chapter 5).

Chapter 1:

General literature review

1.1. INTRODUCTION

Lipids are a group of compounds that are insoluble in water but are soluble in organic solvents. Lipids can be either synthesized within the human body (endogenous lipids) from carbohydrates and proteins or derived from dietary fats (exogenous lipids) (7, 8). Naturally occurring lipids are derived from a wide variety of animal and plant sources, including adipose tissues (visible fat on meats), milk and dairy products, seeds, nuts, eggs and seafood (8, 9). The most common nutritional lipids are edible oils (liquid at room temperature) and fats (solid at room temperature) (7). Common lipid classes include phospholipids (functional lipids), triglycerides (food and stored lipids), esterified and free cholesterol (membrane component and substrate for the synthesis of steroid hormones/vitamin D/bile acids) and free (un-esterified) fatty acids. The functions of fatty acids, both free and as part of complex lipids, include energy storage, maintenance of the structural and functional integrity of all cells/tissue membranes, carriers of fat-soluble substances (fat-soluble vitamins, carotenoids, polyphenols), substrates for biologically active eicosanoids, and post-translational modification of proteins. Stored lipids in adipose tissue serve to provide insulation, help to regulate body temperature, and provide physical protection to internal organs (7, 8). Due to their varied functions, fatty acids play a key role in disease causation and prevention**.**

Dietary fatty acid composition modulates metabolic pathways associated with the occurrence and consequences of the metabolic syndrome (10), setting up the stage for type 2 diabetes and cardiovascular disease. Therefore, a better understanding of the effect of different fatty acids and the mechanisms by which different fatty acids modulate lipid metabolism is crucial.

This chapter review the current knowledge relating to the characteristics of fatty acid, particularly chain lengths of SFA, as well as their beneficial and detrimental effects on lipid metabolism and cardiovascular risk.

1.2. FATTY ACIDS:

The fatty acid are carbon chains with a methyl group at one end of the molecule and a carboxyl group at the other end. They may contain zero to multiple unsaturations (double bonds between their carbon atoms) and are usually grouped based on the number and

position of double bonds in their chains (5). Structurally, they are grouped according to the number of double bond in their carbon chain as saturated, monounsaturated and polyunsaturated fatty acids (5). Fatty acid is represented by its structural formula as Cx: y(n-z); where x = the number of carbon atoms, y = the number of double bonds, and z = the position of the first double bond counting from the methyl end of the molecule (11).

Fatty acids are an important component of phospholipids, diglycerides, monoglycerides, and sterol ester. In addition, fatty acids are also one of the most important components of the triglyceride molecule (Figure 1.1), which consists of three fatty acids esterified to the hydroxyl groups of the glycerol at distinct stereo specific positions, denoted sn-1, 2, and 3 (8, 11). Fatty acids can remain in the free (unesterified) state or be esterified to phospholipids, cholesteryl ester (5).

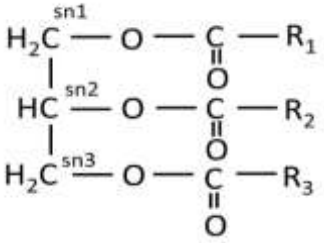


Figure 1.1: General structure of triglyceride. R indicates the fatty acid chain, and sn1 to 3 indicate the position where the fatty acid is located.

1.2.1. Saturated fatty acids

Saturated fatty acids (SFA) contain no double bonds in their carbon chain. Based on their chain length, SFA can be sub-divided into short, medium and long chain saturated fatty acids (7).

a) Short chain SFA (SCFA) are 2 to 4 carbons long (e.g. acetate, propionate, butyrate) and are naturally present in dairy products (7, 12). Short chain SFA are also formed during bacterial fermentation of carbohydrates in the colon.

b) Medium chain SFA (MCFAS) (example in Figure 1.2) are 6 to 12 carbons long (e.g. caproic, caprylic, capric and lauric acid) found in coconut oil, palm kernel oil, and citrus seeds (7).

c) Long chain SFA (LCSFAS) are 14 or more carbons long (e.g. myristic, palmitic and stearic acid) and are found in palm oil, lard, beef tallow, suet, and butter (7, 9).

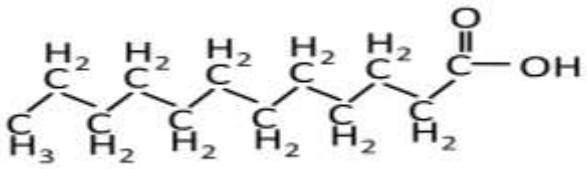


Figure 1.2: Lauric acid ($C_{12}H_{24}O_2$) structural formula.

1.2.2. Monounsaturated fatty acids

Monounsaturated fatty acids (MUFA) contain one double bond in their structure. According to their geometric position, naturally occurring fatty acids can be cis [e.g. oleic acid, Figure 1.3] or trans [e.g. elaidic acid] (13). Rich sources of MUFA with a cis double bond are canola oil, avocado, almonds, cashew nuts, olive oil and peanuts (7, 9).

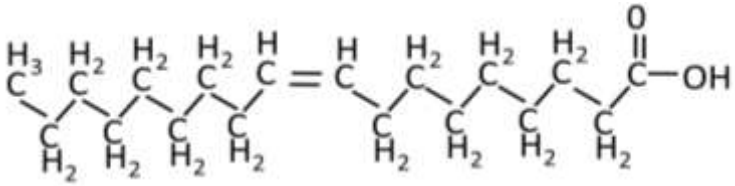


Figure 1.3: Oleic acid ($C_{18}H_{34}O_2$) strutural formula.

1.2.3. Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFA) contain two or more double bonds in their structure. PUFA may belong to either the omega-3 or the omega-6 family.

a) Omega-6 polyunsaturated fatty acids (n-6 PUFA) are those with the first double bond located at the sixth carbon atom from the methyl end, e.g. linoleic acid (LA) and arachidonic acid (AA). Good sources of LA are soybean, sunflower, corn, safflower and cottonseed oil, while AA may be found in animal products (14) or synthesised by the human body from linoleic acid (15).

b) Omega-3 polyunsaturated fatty acids (n-3PUFA) are those with the first double bond located at the third carbon atom from the methyl end, e.g. α-linolenic acid (ALA, Figure 1.4). ALA is found in walnuts, canola oil, linseed and chia seed while long-chain n-3PUFA (eicosapentaenoic acid, EPA, docosapentaenoic acids, DPA and docosahexaenoic acid, DHA) are found in fish and other marine sources (7, 16).

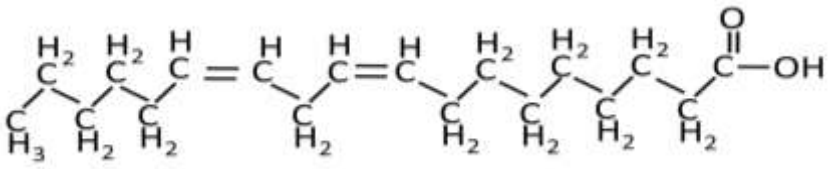


Figure 1.4: Linoleic acid ($C_{18}H_{32}O_2$) strutural formula.

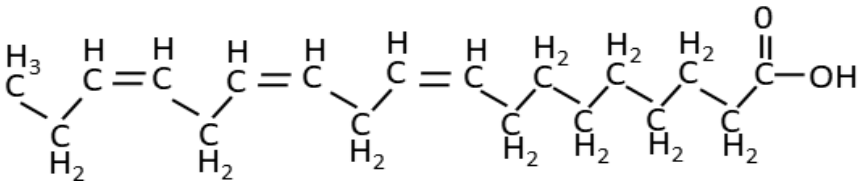


Figure 1.5: α- Linoleic acid ($C_{18}H_{30}O_2$) strutural formula.

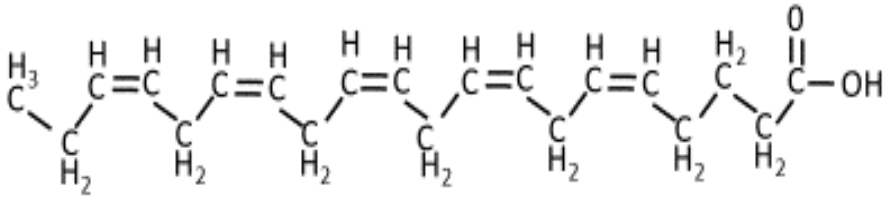


Figure 1.6: EPA ($C_{20}H_{30}O_2$) strutural formula.

1.2.4. Trans fatty acids

Trans-fatty acids (TFA) are unsaturated fatty acids with double bonds in trans configuration. TFA are produced naturally in the stomach of ruminants and by industrial partial hydrogenation of vegetable oils (17). Thus, TFA are found in processed foods (e.g. margarines, shortenings, peanut butter, sauces, soups, baked goods) and in animal products from ruminants (e.g. dairy, beef and lamb meats) (18).

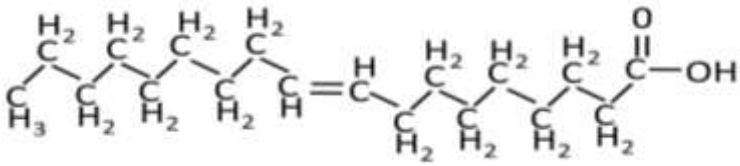


Figure 1.7: Elaidic acid ($C_{18}H_{34}O_2$) strutural formula.

1.3. ENDOGENOUS AND DIETARY FATTY ACIDS

Diet is one of the important factors which control the tissue fatty acid composition of an individual (19). Fatty acids supposed to be exclusively of exogenous origin such as n−3 PUFA, n−6 PUFA, *trans*, and odd-numbered chain length fatty acids, could provide dietary intake estimates of usual food intakes (20). On the other hand, fatty acids such as SFA and MUFAs in plasma as well as erythrocytes, which can be synthesized endogenously from dietary carbohydrates, might not reflect usual dietary fatty acid intake (21). Fatty acids can be quantified in several tissues and blood fractions for e.g. plasma or serum, erythrocytes, and adipose tissue. The adipose tissue lipids of healthy adults are made up 99% fatty acids, 0.3% cholesterol and less than 0.1% phospholipids. The major fatty acids in the subcutaneous adipose tissue and in plasma are oleic, palmitic and linoleic acid. Adipose tissues are considered as a biomarker for long-term dietary intake of fatty acid (21); however, plasma and erythrocytes as biomarkers of fatty acid intake are frequently used, probably that is because collecting and allocating the blood sample for several laboratory analysis is relatively easy (22-24). The half-life of erythrocytes (120 days) is much longer as compared to plasma lipoproteins. Plasma fatty acids, compared with erythrocyte fatty acids, reflect short-term consumption of dietary fatty acid due to more sensitivity to recent consumption as well as a faster turnover rate (25). Fatty acid incorporation as fat depot as triglyceride in response to sources of fatty acids in the human body and the assessment of its effect to patho-physiopathological processes, for example the control of fat deposition and of body weight, requires investigation. Accurate examination of consumption of fat is required to study the relationship between diet and disease risk, however the way of measuring individual consumption of fat quality by dietary assessment is difficult (25). It is well known that some fatty acids such as medium-chain triglycerides are deposited less in the adipose tissue and are quickly metabolised, making them a important tool for body weight control (26).

The fatty acids present in body tissue and fluids are derived directly from the dietary sources or by endogenous synthesis in the body (11). Two important enzymes used in *de novo* fatty-acid synthesis includes acetyl coenzyme A carboxylase (ACC) and fatty-acid synthase (FASN). Fatty acids are formed from carbohydrates and proteins, predominanlty in the liver and the adipose tissue, when an excess of energy as carbohydrate or protein is consumed. The hydrolysis of carbohydrate yields glucose, which is converted to pyruvate and is subsequently converted to acetyl CoA. Acetyl-CoA carboxylase converts acetyl-

CoA to malonyl-CoA, which is converted to palmitate by fatty acid synthase. Palmitate is the precursor of other long-chain fatty acids. It may be lengthened and desaturated to other fatty acid. Proteins that are broken down into amino acids are deaminated and converted to pyruvates. The switch between fatty acid synthesis and its oxidation is regulated by malonyl-CoA. The overproduction of malonyl-CoA due to excess energy intake inhibits the oxidation of fatty acids and the reduction in malonyl-CoA levels lead to an increase in fatty acid oxidation. The excess malonyl-CoA is then reverted for fatty acids synthesis. Triglyceride synthesized in adipose tissue serves as the major energy storage, while triglycerides produced in the liver are substrates for the production of very low-density lipoprotein (VLDL) particles, which are secreted into the circulation (1, 5, 27)

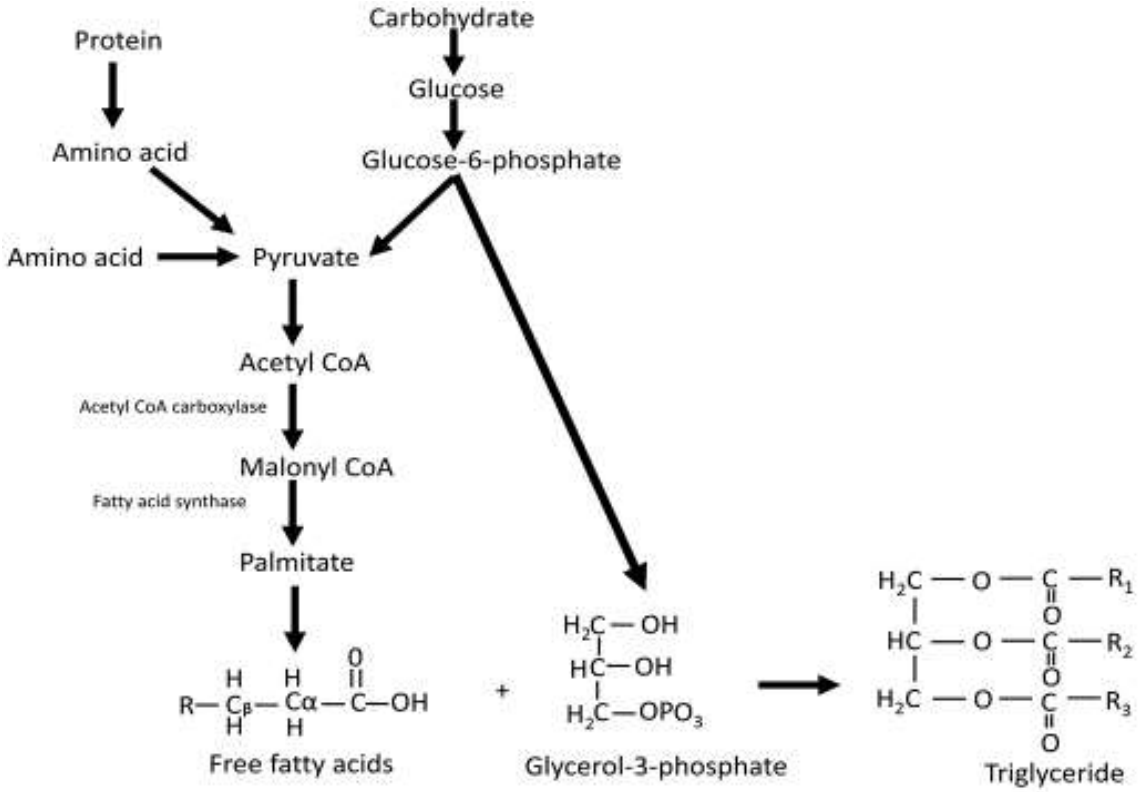


Figure 1.8: Schematic diagram of fatty acid biosynthesis from proteins and carbohydrates. Briefly, proteins and carbohydrates are converted to pyruvate, palmitate and then to free fatty acids. Carbohydrates may also be converted to glycerol-3-phosphate, combine with free fatty acids and form new triglyceride. R, fatty acid chain; CoA, coenzyme A. Adapted from Sul and Smith (1) and Whitney and Rolfes (5).

1.4. LIPOPROTEIN METABOLISM

Ingested fats are cleaved by enzymes, absorbed in the small intestine, transported into the lymphatic system and flows into blood. The composition of dietary lipids could regulate lipids and lipoprotein metabolism and may confer cardiovascular benefit. The importance of regulating lipoprotein metabolism in improving plasma lipids and CVD risk has been investigated by many authors (28, 29). Few studies have investigated the effect of dietary SFA consumption on lipoprotein metabolism in human. Chain length of the SFA may also have differential effects on lipid and lipoprotein metabolism. However, several studies have focused on the study of the effect of *n*-3 PUFA on lipoprotein metabolism. This knowledge demonstrates the significance of regulating lipoprotein metabolism in order to improve the plasma lipid profile and reduce the risk of CVD (30, 31). Increased low-density lipoprotein (LDL) and triglycerides are linked with increases risk for atherosclerosis; however, an increase in high-density lipoprotein (HDL) has a positive effect on cardiovascular health (32, 33).

1.4.1. Structure and Functions

Lipids are insoluble in water and therefore are transported as lipoprotein complexes, composed of various lipid classes (e.g. cholesterol, triglycerides, and phospholipids) and proteins known as apolipoprotein. Lipoproteins are complex particles that have a hydrophobic core consisting of liposoluble vitamins, cholesterol esters and triglycerides. This hydrophobic core is surrounded by a hydrophilic coating containing free cholesterol, phospholipids, and apolipoproteins (34, 35). Plasma lipoproteins can be divided based on diameters, lipid composition (lipid and apolipoprotein content), density, floatation characteristics and function. There are five main classes of lipoproteins: chylomicrons, VLDL, intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL); presented in the order of increasing density and decreasing diameter (7, 8). Based on lipid composition, lipoproteins are classified into triglyceride-rich lipoproteins, which consist of chylomicrons and VLDL; and cholesterol-rich lipoproteins, which are LDL and HDL (35). Cholesterol can be synthesized by nearly all cells in the body, and 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase is the rate limiting step for cholesterol synthesis. Cholesterol is an essential structural component of cell membranes and is a precursor of steroid hormones and of bile acids. Due to its hydrophobicity, cholesterol travels in the blood transported

in molecules called lipoproteins (36). Additionally, lipoprotein subclasses have different apolipoproteins bound to the lipid surface, which determines their function. Apolipoproteins are useful in lipoprotein metabolism to maintain the structural integrity, solubility of lipoproteins, receptor recognition and the regulation of certain enzymes (5, 37). Among the major human apolipoprotein classes, apolipoprotein B (apo B) is an important protein constituent of chylomicrons (apo B-48), LDL (apo B-100), and VLDL (apo B-100); while apolipoprotein A-I (apo A-I) is the major protein constituent in HDL. The important role of lipoprotein particles is to transport triglyceride (TG) and cholesterol in the circulation. In the forward lipid transport system, triglyceride-rich chylomicrons and VLDL particles deliver dietary and hepatic triglycerides to peripheral tissues, while LDL particle delivers cholesterol to peripheral tissues. However, in the reverse cholesterol transport system, HDL particles mediate the transport of cholesterol from peripheral tissues to the liver for catabolism. Lipoprotein metabolism can be divided into the exogenous, endogenous pathway, and the reverse cholesterol transport pathways (38-41).

1.4.2. The Exogenous Pathway

The process of lipid digestion starts in the mouth, where some lipids break down into diglycerides by the lingual lipase. Emulsification of lipids occurs in the stomach, where small amounts of lipids is hydrolysed by the action of gastric lipase. However, most lipid digestion occurs in the duodenum and jejunum of the small intestine. Bile salts emulsify lipids arriving in the duodenum, greatly increasing the surface area available to the lipases where pancreatic and intestinal lipases breakdown lipids (35, 42). Fatty acid chain length may influence the digestion efficiency of fatty acids; triglycerides of SCFA and MCFAS are more efficiently hydrolysed compared to triglycerides of LCSFAS. This is due in part to the higher water solubility and higher mobility at the emulsion-water interface. Lingual, gastric and pancreatic lipases have greater activity against short and medium-chain triglycerides than long-chain triglyceride in the stomach generating fatty acids and monoglycerides prior to release into the duodenum (5, 43). Most of the SCFA and MCFAS are readily absorbed by passive diffusion in their free form. Inside the enterocyte, SCFA and MCFAS exhibit lower affinity for fatty acid binding protein and therefore pass through the enterocytes without being esterified. Therefore, SCFA and

MCFAS are bound to albumin and transported as non-esterified fatty acids (NEFAa) via the portal bloodstream to the liver (43, 44).

However, following digestion and absorption into the intestinal mucosa from the lumen, LCSFAS are re-esterified into triglycerides, reassembled into large chylomicron particles in the Golgi complex of intestinal cells. Chylomicrons are carried in the lymphatics from the intestine and enter the circulatory system via the thoracic duct (5, 44). The lipid composition of chylomicrons consists of approximately 90% TG, with the remainder consisting of cholesterol ester, free cholesterol, phospholipids, and protein. Apo B48, the main apolipoprotein of chylomicron which in humans is synthesized by the intestine, is essential for the assembly and secretion of chylomicrons**.** Chylomicrons also carry apo A-I and apolipoprotein A-IV (apo A-IV) and after secretion acquire apolipoprotein C-III (apoC-III), and apolipoprotein E (apoE) from the circulation (45, 46). Chylomicrons appear in the bloodstream after ingestion of a meal with peak concentration occurring between 3-6 hours after that meal. Therefore, fasting levels of chylomicrons and apoB-48 is barely detectable in most individuals. Upon entering the circulation, lipoprotein lipase hydrolyses the TG within the chylomicrons into free fatty acid and monoglycerides on the surface of endothelial cells, generally in adipose and muscle tissue. These hydrolytic products are either utilized as an energy source by peripheral tissues or stored in adipose tissue. When more than 90% of the chylomicron-TG hydrolysis is complete, the chylomicrons, now called chylomicron remnants, are released back into the blood circulation (5, 45, 47). Both chylomicron and chylomicron remnants are taken up by the liver through the LDL-receptors (LDL-r); and TG is again hydrolyzed and stored in the liver; or the TG is repackaged into VLDL and released into the circulation. ApoE is the predominant protein that allows the chylomicron remnant to be recognised and removed by the LDL-r in the liver. Upon the binding of chylomicron remnants to LDL-r, chylomicron remnants are rapidly internalized by the cells and the particles are subsequently degraded in lysosomes. Chylomicrons are atherogenic lipoproteins as they can be trapped in the artery wall and take part in foam cell formation. Thus, it is of considerable significance that these chylomicron remnants are efficiently removed from the circulation (45, 46, 48). Once transported to the liver, SCFA & MCFAS enter the β-oxidation pathway in liver mitochondria independent of the carnitine transport system and undergo preferential oxidation. At a cellular level, LCSFAS require a carnitine shuttle to be transported to the mitochondria (35, 49).

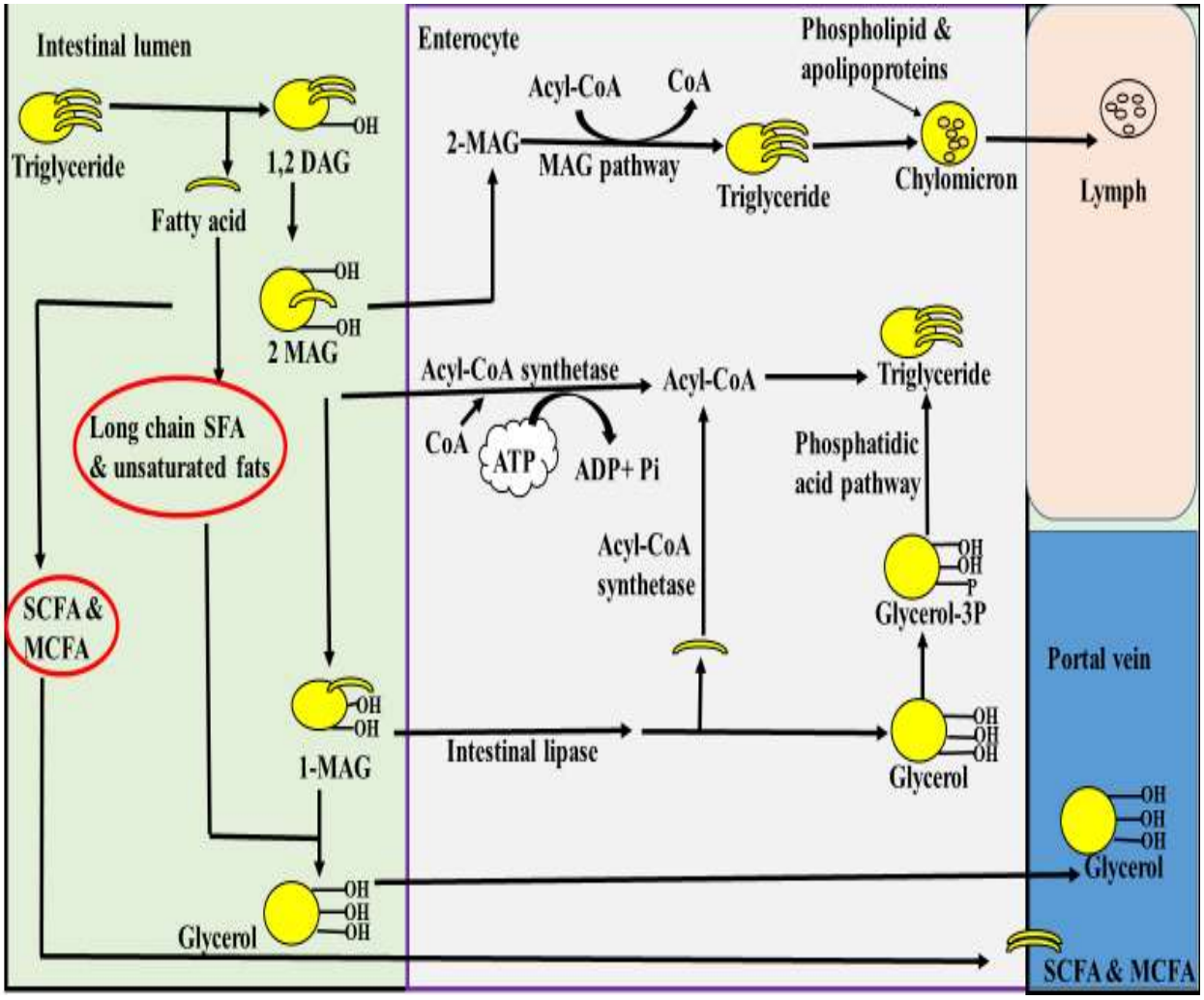


Figure 1.9: Digestion and absorption of short, medium and long-chain saturated fatty acid. Briefly, pancreatic and intestinal lipase break down dietary triglyceride into fatty acids and monoglycerides. SCFA and MCFAS move out of micelles and enter cells by diffusion. Absorbed LCSFAS combine with cholesterol and protein in the intestinal cells to form chylomicrons. SCFA, Short-chain fatty acid; MCFAS, Mediuma-chain fatty acid; LCSFAS, Long-chain saturated fatty acid; 1,2 DAG, 1,2 di-glyceride; 1 MAG, 1 monoglyceride; 2 MAG, 2 monoglyceride; CoA, coenzyme A; ATP, Adenosine triphosphate; ADP, Adenosine diphosphate; Pi, phosphate. Adapted from Dominiczak and Baynes (3).

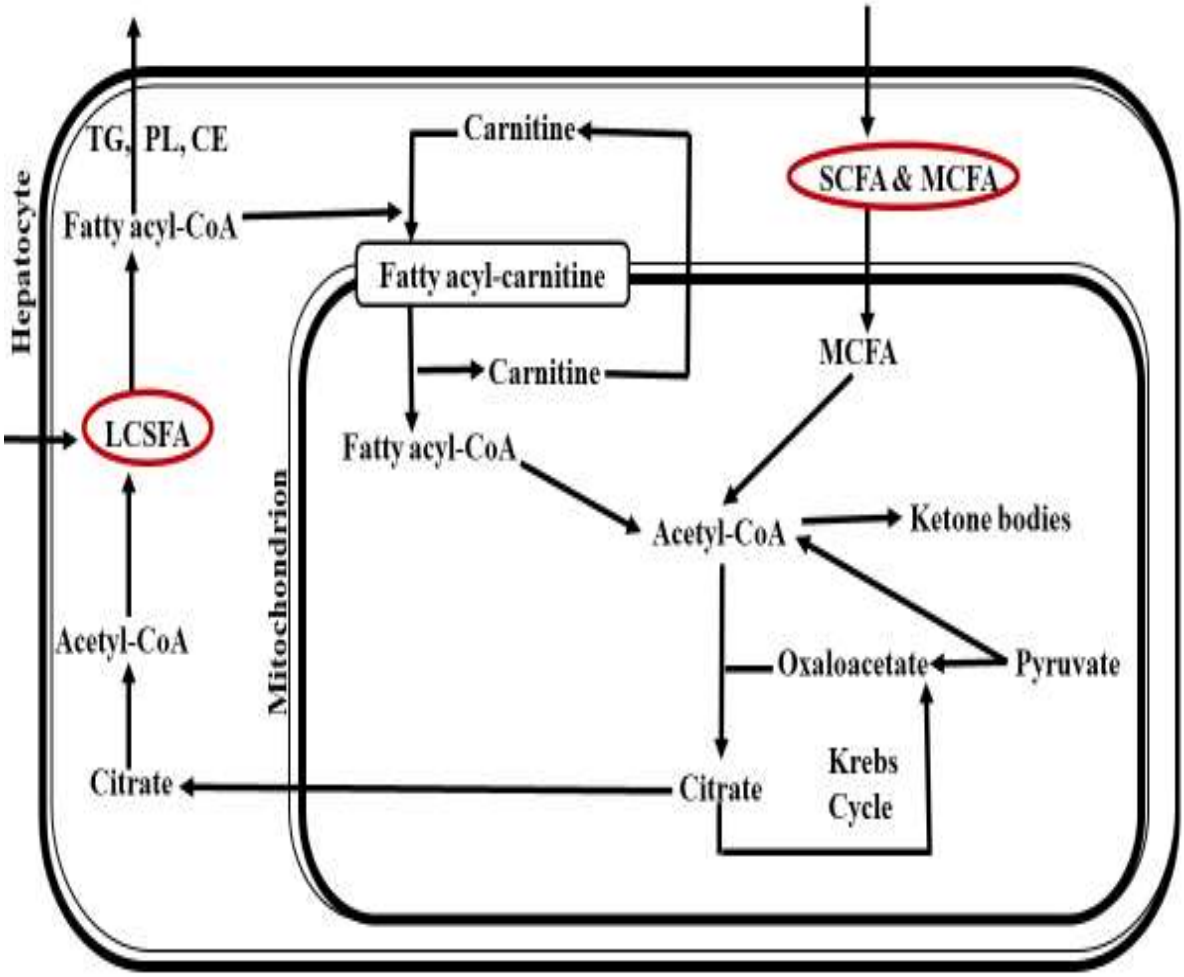


Figure 1.10: Metabolism of fatty acids in the liver and mitochondria. Briefly, SCFA and MCFAS cross the double mitochondrial membrane very rapidly, and do not require the presence of carnitine as do LCSFAS. Excess acetyl-coA, then follows metabolic pathways, both in the mitochondria (krebs cycle) and in the cytosol, resulting in the production of ketones. TG: Triglyceride; PL: Phospholipid; CE: Esterified cholesterol; SCFA: Short chain fatty acid; MCFAS: Medium-chain fatty acid; LCSFAS: Long-chain saturated fatty acid; CoA, coenzyme. Adapted from Bach and

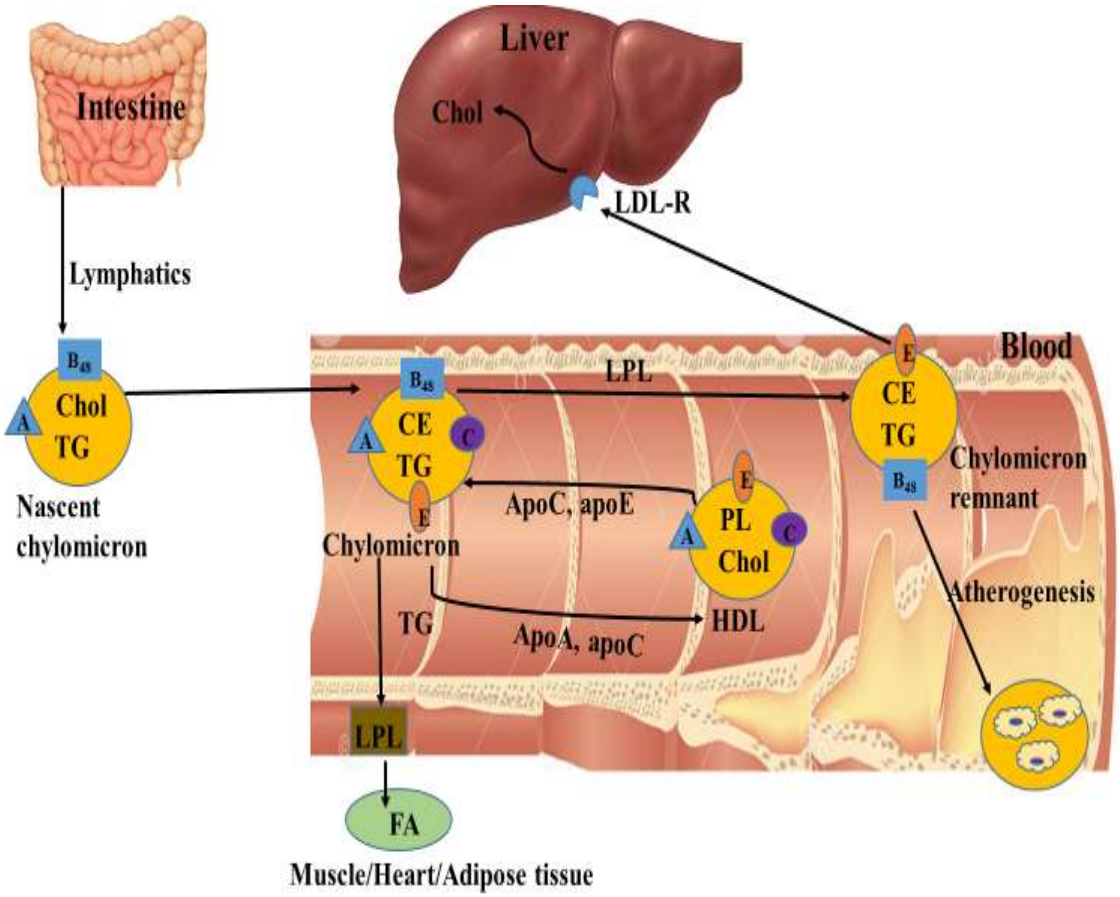


Figure 1.11: Metabolism of chylomicron. Briefly, nascent chylomicrons produced in enterocytes are transferred to bloodstream through the lymphatic system. In bloodstream, chylomicron acquire apoC and apoE from HDL. In capillaries, LPL hydrolyse chylomicrons and fatty acids are delivered to extrahepatic tissues. ApoC and apoA are transferred to HDL, while apoE remain linked with chylomicron remnant. Chylomicron remnants are taken up by the LDL-receptor. TG: triglyceride; PL: phospholipid; Chol: cholesterol; A: apolipoprotein A; B48; apolipoprotein B-48; C; apolipoprotein C; E; apolipoprotein E; LPL: Lipoprotein lipase; FA; fatty acid; LDL-R; low density lipoprotein receptor; HDL; high density lipoprotein. Adapted from Bersot (4), Vaziri and Norris et al. (6).

1.4.3. The Endogenous Pathway

The liver has the greater capacity for storage and maintenance of lipid homeostasis during the transition from fed to fasted states than the intestine. VLDL triglyceride (VLDL-TG) are assembled in the liver from fatty acids that are either synthesized de novo, extracted from the circulation as nonesterified fatty acids, or recycled from lipoprotein remnants cleared by hepatic receptor (5, 27). VLDL is secreted from the liver, where apo B-100, phospholipids, and a small amount of free cholesterol form the surface of VLDL; and TG and cholesterol ester form the core of the particle. Nascent VLDL released from the liver contain apo B-100, as well as some apoE and apoC secreted from the hepatocytes. Apolipoprotein E plays a significant role in the pathway for the synthesis and secretion of VLDL. Upon its entry into the bloodstream, VLDL acquires additional apoE and apoC by transfer from circulating plasma HDL (50-52). The hydrolysis of TG components of VLDL into free fatty acids and glycerol by capillary LPL and loss of apoE and apoC leads to the formation of progressively smaller lipid poor VLDL (known ad VLDL remnants) or IDL, and ultimately LDL particles (48, 52). In humans, the TG content of VLDL is also reduced by the action of cholesteryl ester transfer protein (CETP); CETP transfers TG from VLDL to HDL in exchange for cholesteryl esters (53). The competition between VLDL and chylomicrons for the same lipolytic and receptor-mediated uptake pathways throughout the postprandial state, in part, leads to the accumulation of triglyceride rich lipoproteins. VLDL is much smaller in size than CM, therefore each particle presumably interacts with comparatively fewer LPL molecules, resulting in a slower rate of VLDL-TG hydrolysis (54). In healthy subjects, adipose tissue lipolysis is higher in the fasting state compared with the fed state due to low insulin level, leading to higher NEFA secretion and thus an enhanced VLDL-TG production of the liver (55). LDL particles are formed from VLDL particles or synthesized by the liver. The main apolipoprotein of LDL is apoB100 and therefore measurement of apoB100 may also provide an estimation of circulating LDL particle concentration. LDL particle size and concentration may be influenced by alteration in VLDL particle concentration and VLDL de-lipidation (56). A number of studies indicate that dietary fatty acids can modulate the size, concentration and distribution of LDL particles. Many recent studies have demonstrated that, at a given LDL cholesterol concentration, individuals whose LDL particles are predominantly small, dnese can be at a higher risk of coronary heart disease (CHD) compared with those with larger-size LDL particles (57). Therefore, LDL particle

size appears to be a good predictor of cardiovascular disease and progression of coronary artery disease (58). SFA increase the levels of LDL particles, reducing the expression of hepatic LDL-r activity and thereby reducing the LDL-r mediated uptake of LDL particle from circulation (59, 60)

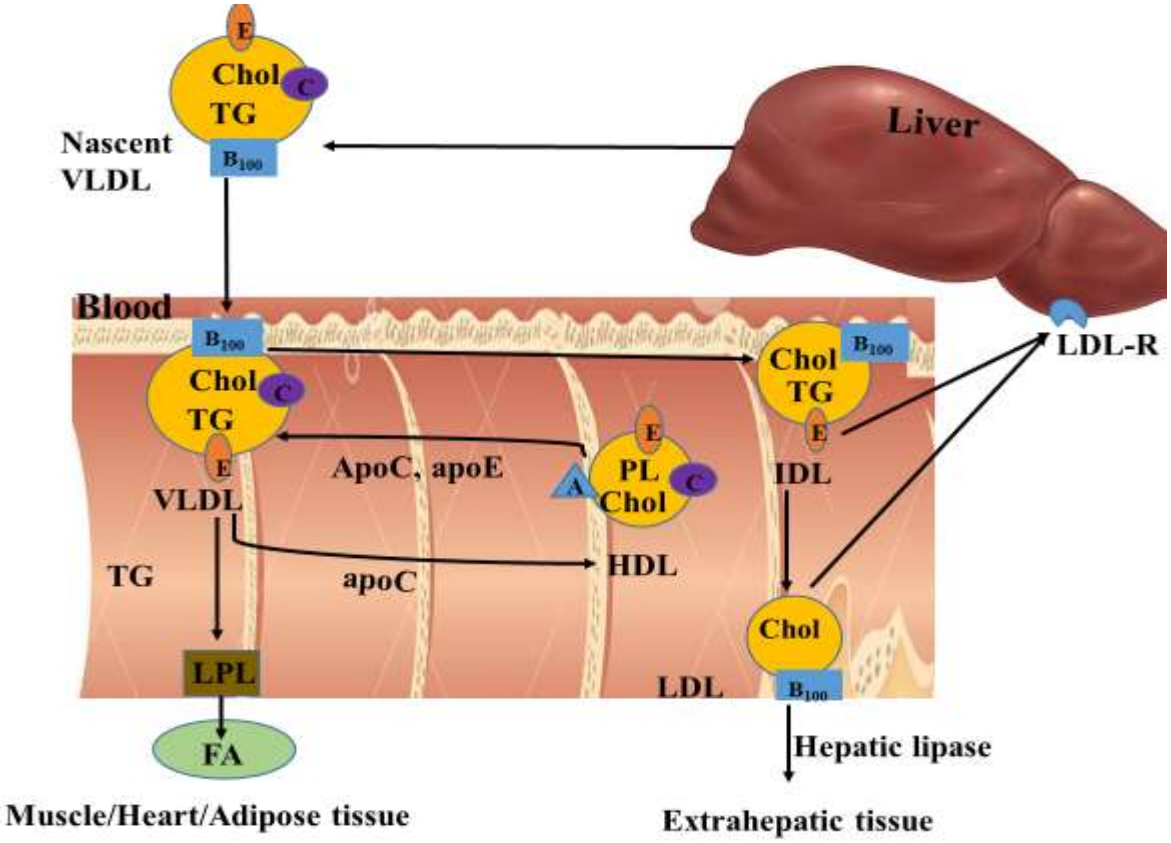
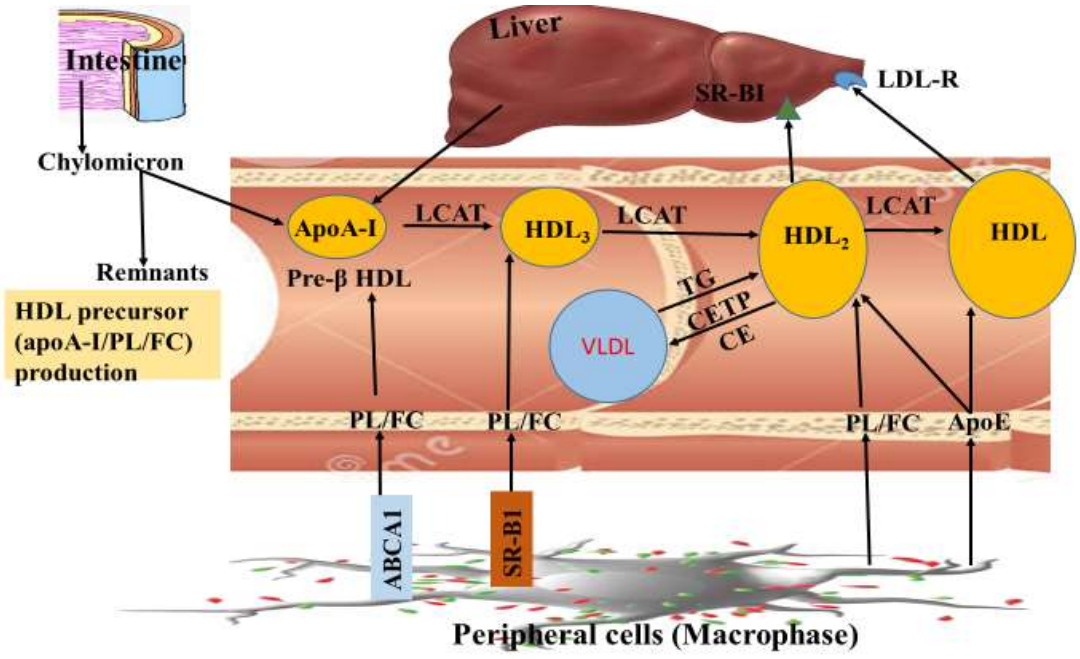


Figure 1.12: Metabolism of VLDL. Briefly, nascent VLDL are produced by the liver. In bloodstream, VLDL acquire apoCs and apoE from HDL. In capillaries, LPL hydrolyse chylomicrons and fatty acids are delivered to extrahepatic tissues. Loss of triglyceride from VLDL leads to the formation of IDL and apoCs are transferred to HDL. Further loss of triglyceride by hepatic lipase from IDL leads to formation of LDL. LDL are removed from the circulation via apoB100 mediated endocytosis by LDL receptor. TG: triglyceride; PL: phospholipid; Chol: cholesterol; A: apolipoprotein A; B48; apolipoprotein B-48; C; apolipoprotein C; E; apolipoprotein E; LPL: Lipoprotein lipase; FA; fatty acid; LDL-R; low density lipoprotein receptor; HDL; high density lipoprotein; IDL, intermediate density lipoprotein. Adapted from Bersot (4).

1.4.4. HDL metabolism and reverse cholesterol transport

Most peripheral tissues and cells cannot catabolize cholesterol, therefore reverse cholesterol transport is important in regulating cellular cholesterol homeostasis. The continuous transfer of cholesterol through LDL to peripheral cells is balanced by reverse cholesterol transport pathway, a multi-step process where there is the net movement of cholesterol from peripheral tissues back to the liver for excretion. Removal of cholesterol from macrophages is important because cholesterol-loaded macrophages results in the formation of foam cells. apo A-I is the main apolipoprotein in HDL and is synthesized by the liver and the intestine. In humans, deficiency in apo A-I has been linked with low concentrations of HDL and coronary heart disease (61, 62). The liver and intestine synthesize and secrete nascent HDL or pre-β HDL particles containing mainly apo A-I and phospholipids (63, 64). Lipidation of nascent HDL occurs primarily by ATP-binding cassette sub-family A member 1 (ABCA-1) transporter mediated efflux from extrahepatic cells and LPL-mediated lipolysis of triglyceride rich lipoproteins to form HDL_3 particles (65, 66). In the nascent HDL, free cholesterol is esterified to cholesteryl ester by the action of lecithin-cholesterol acyltransferase (LCAT), carried on HDL_3 particles, and activated by apo A-I. Hydrophobic cholesterol esters are retained in the HDL core, resulting in the formation of mature, spherical HDL_2 and HDL particles (67). In the core of HDL, the cholesterol ester via interaction with scavenger receptor class B type 1 (SR-B1) receptors (highly expressed in the liver) can be taken by the liver for secretion into the bile via, after which the resultant lipid-free apo A-I particles are recycled into the circulation to acquire more free cholesterol from the plasma membrane of cells in peripheral tissues (64, 68, 69).


Intestine
Chylomicron
Remnants
HDL precursor
(apoA-I/PL/FC)
production
Liver
SR-BI
LDL-R
ApoA-I
LCAT
HDL3
LCAT
HDL2
LCAT
HDL
Pre-β HDL
TG
CETP
CE
VLDL
PL/FC
PL/FC
PL/FC
ApoE
ABCA1
SR-B1
Peripheral cells (Macrophase)

1.5. POSTPRANDIAL LIPID METABOLISM

The postprandial state can be defined as a physiological state after meal consumption involving absorption, transport and assimilation of ingested dietary fats. This postprandial phase usually lasts 6-8 hours in normal individuals. A study of fasting lipid profile assesses the endogenous phase of lipid metabolism, whereas the studies in the postprandial state are essentially, although not completely, looking at the exogenous lipid metabolism pathway (70). Our knowledge of the relationship between lipid/lipoprotein metabolism and cardiovascular disease is based on fasting blood measurements. Although measurement in the fasting state remains the basis of clinical assessment and decision making regarding hypolipidemic interventions, it is important to know that people spend the majority of time in a nonfasting, postprandial state (71) . Most people consume three or more meals over the course of the day, and each meal contains 20 to 70 g of fat. Plasma TG peak 3-5 hours after the meal, before it returns to a post-absorptive state (70, 72). Each of the subsequent meal in daily life is most probably consumed before plasma lipid concentrations have returned to post-absorptive state from the lipaemic state resulting from the previous consumption. Therefore, people in most of their lifetime are in a postprandial state, with a continual fluctuation in the degree of postprandial lipemia (73). Measurement of the postprandial lipid response is challenging, and it is, therefore, more complex to examine the cardiovascular disease (CVD) risk factor linked with postprandial lipemia compared to the fasting condition. However, there is strong evidence to suggest that future studies to treat lipids related atherogeneity should include parameters related to postprandial lipemia (74). Elevated postprandial TG concentrations can reflect impaired postprandial lipid metabolism and delay in clearance of triglyceride rich lipoprotein (TRL) from the circulation. The increased TRL and the increased residence time in the circulation may increase CETP mediated exchange rate between TRL and esterified cholesterol from HDL and LDL. This leads to an increased preponderance of small-dense LDL and small HDL particles, a lipoprotein profile known as the atherogenic lipoprotein phenotype, which is associated with increased CVD risk (72). About 6-8 hours after the meal is consumed, there is increased clearance of circulating chylomicrons from the circulation. Several studies have employed 6-8 hours postprandial challenge time, since this has proved necessary for triglyceride levels to return to post-absorptive state in healthy subjects (73, 75). However, this time may need to be extended in patients with known triglyceride disturbances. The most common

technique used by several investigators to calculate the area under the curve is trapezoidal rule. This, taken with the growing body of evidence associating elevated levels of postprandial TG and TRL with CVD risk, suggests that dietary interventions, especially dietary fat, which can lower postprandial lipemia might help to decrease overall CVD risk (75, 76). Several studies have shown that dietary fats modulate blood lipid profile and CVD risk. From those studies, many researchers have investigated short to long term metabolic effects of fats measured in fasting state in human subjects. Therefore, future studies are needed to compare the association of fasting and non fasting lipid profiles and cardiovascular outcomes in the same individuals (77).

1.6. DIETARY FATS & BLOOD LIPIDS:

Dietary fats have been shown in several scientific publications to modulate blood lipid levels and lipoprotein profiles and CVD risk (59). Dyslipidemia, characterized by elevated concentrations of TC, LDL-C and TG, and reduced levels of HDL-C, is an independent and powerful risk factor for CVD (78). In 2011-2012, one third of the Australian adult population had increased LDL-C levels and 23% had reduced HDL-C levels compared to the reference value (79). Nutritional and lifestyle modifications are important for the prevention/treatment of abnormal blood lipid levels to lower CVD risk. Current dietary guidelines focus on dietary fats and recommend reductions in saturated and TFA intake, and an emphasis on consumption of MUFA and PUFA. However, the dietary recommendation on reducing SFA intake without considering the replacement nutrient might not produce substantial health benefits (80, 81). In addition, reduction in SFA also lowers HDL-C levels; therefore, blanket recommendation to reduce SFA intake in the diet may not be appropriate. Therefore, studies suggesting that the food containing SFA and kind of SFA are more important parameters of their lipid levels modulation (82) needs to be considered. In addition, studies investigating the mechanisms of action of these fatty acids in the human physiology is, therefore, crucial in contemporary cardiometabolic studies.

Existing scientific evidence on the mechanisms of action by which dietary fatty acids modulate circulating blood lipid concentrations is supported by data from several animal models. Fernandez et al. (59) have shown that dietary fats regulate circulating LDL-C levels by affecting LDL-r activity, protein and mRNA abundance. In addition,

dietary fatty acids regulate the plasma cholesterol level indirectly by regulation through families of several transcription factors: peroxisome proliferator activated receptors (PPARs), liver X receptors (LXRs), hepatic nuclear factor-4 (HNF-4) and sterol regulatory element binding proteins (SREBPs) (59). Dietary SFA increase LDL-C levels by inhibiting LDL-receptor activity and promoting LDL production rate (83, 84). The LDL-r helps to control cholesterol levels, therefore, any decrease in LDL-r activity leads to an increase in cholesterol in circulation. SFA are recognised to inhibit ACAT (the rate-limiting enzyme which regulates the esterification of cholesterol). Study have demonstrated that the activity of LDL-r is inhibited in humans and animal models by SFA diet (particularly long chain SFA) (85). A number of in vitro and animal studies have shown that dietary fats modulate in the liver the activity of LDL-r by cholesteryl ester and unesterified cholesterol pools (86-88). PUFA have been demonstrated to increase LDL-r activity, protein and mRNAabundance via modulation of membrane fluidity of hepatocyte (59). Since the majority of the existing evidence on the mechanism of dietary fatty acids on modulating blood lipid concentration is dependent on animal or cellular model studies, further human clinical trials are warranted to elucidate the exact mechanisms by which dietary fats modulates blood lipid levels.

1.6.1. Unsaturated fats

Recently, trans fatty acids (TFAs) have been linked to increased risk for CVD, since several scientific studies have demonstrated that TFA increase plasma LDL-C and lipoprotein (a) and decrease plasma HDL-C levels (89). It has been demonstrated that consumption of ruminant sources of TFA from natural sources, in particular, conjugated linoleic acid (CLA), may exert different biological effects compared with industrial sources of TFA. However, intervention trials review addressing a similar question have demonstrated that TFA from the industrial hydrogenation (n = 29 studies), ruminant sources (n = 6 studies), and CLA (n = 17 studies) all increased the LDL:HDL ratio to a nearly similar extent (90). On the other hand, cis isomers of MUFA reduce LDL-C and increase HDL-C levels (91-93). PUFA of the n-6 PUFA family have been shown to be effective in lowering circulating TC and LDL-C levels without affecting TG or HDL-C levels (59). Conversely, it has been postulated that an increased dietary intake of n-3 PUFA, particularly of marine origin (eicosapentaenoic acid and docosahexaenoic acid)

are potent lipid TG lowering agents (94). This effect may in part were mediated by reducing hepatic TG synthesis and VLDL secretion and by increasing TG catabolism through up-regulation of LPL enzyme (94). Therefore, the most compelling evidence that increasing the dietary intake of n-3 PUFA improves several CVD risks is partly mediated by its potent TG lowering effects. In addition, n-3 PUFA possess mild LDL-C and HDL-C raising effects while shifting small, dense LDL to large, buoyant and less atherogenic particles (95, 96).

1.6.2. Saturated fats

The positive linear relationship between SFA intake and total and LDL cholesterol levels (lipid hypothesis) has influenced many authorities and countries to introduce dietary guidelines to restrict the intake of SFA in the general population; many authorities and countries have set an upper limit of 10-11% of energy from dietary SFA (80). However, recent findings have called into question the presumed association between dietary SFA per se with CVD in part due to the need to consider replacement of SFA with different macronutrients (77). Replacement of dietary SFA by TFA increases LDL-C, decreases HDL-C levels, and increases the TC/HDL-C ratio (41, 89). Similarly, replacement of dietary SFA with carbohydrates (CHOs) in the diet has been associated with either no improvement in CVD or even increased CVD risk (80, 97).This may be attributable to atherogenic lipid abnormality, a common trait characterized by increased TG, reduced HDL cholesterol (HDL-C) levels, and increased levels of small, dense LDL (sdLDL) particles (80, 97). Notably, findings from prospective cohort studies demonstrate significant and independent link between small, dense LDL and elevated CVD risk (98). Therefore, in the context of the metabolic syndrome, lowering of SFA consumption may be less crucial for lowering CVD risk in humans than the limitation of dietary carbohydrates, particularly refined carbohydrate and sugar (80, 97). Replacement of SFA with MUFA or ω-6 PUFA decreases total, LDL, as well as HDL-C levels. People in the Mediterranean region consume reasonably large quantities of olive oil (MUFA) and where CHD rates are relatively low (99). The Mediterranean diet is reported as one of the important studied diets for improved heart health (100, 101). The Mediterranean diet is a plant-based pattern, which consist of monounsaturated fats from olive oil, vegetables, legumes, fruits, cereals (preferably as whole grain), nuts and moderate consumption of

fish and shellfish, white meat, eggs and red wine and minimal amount of red meat and dairy products (100, 101). Epidemiological study have demonstrated that together with doing regular physical activity and avoiding smoking, more than 80% of coronary heart disease, 70% of stroke, and 90% of type 2 diabetes can be avoided by chosing healthy food habit consumption that are consistent with the traditional mediterranean diet meal plan (102). Observational and randomized controlled trial have shown that the mediterranean diet improves blood lipid levels as well as primary cardiovascular disease outcomes such as death and events (103).

To date, however, epidemiological and clinical trial findings provide evidence that replacement of SFA with PUFA, particularly n-3 PUFA, may provide cardiovascular benefit (104, 105). Therefore, emphasis on lowering SFA consumption without considering the replacement nutrients for cardiometabolic disease may not result in overall health benefits.

Reduction of SFA in the diet has been shown to reduce HDL-C levels. Three randomized clinical trials have demonstrated that diets providing 6%–7% of calories from SFA achieved a LDL-C reduction of 9%–11% as well as HDL-C reduction of 7%–11% (106-108). Similarly, another randomized clinical trials have shown that diet providing 4% calories from SFA achieved a 8.6% reduction in LDL-C accompanied by 10% reduction in HDL-C levels (109). Despite the inverse relationship between plasma HDL-C and heart health is widely known, there is possibility that patients with low HDL-C do not develop symptomatic CVD, and vice versa (110). Epidemiological study has demonstrated a link between SFA consumption and coronary heart disease (CHD) mortality; lowering of SFA intake could prevent the prevalence of CHD (111). However, a meta-analysis study by Siri-Tarino *et al* (112) demonstrated no link between SFA consumption and CHD. Individual SFA at a high fat energy intake modulates plasma lipids differently; myristic acid (C14:0) is the most hypercholesterolemic SFA compared to lauric (C12:0) or palmitic (C16:0) acids, whereas stearic acid (C18:0) has a neutral effect (113, 114). However, fats are not consumed in isolation; therefore, recent scientific studies have suggested the importance of food sources of SFA on lipid modulating potential. Therefore, although the individual dietary SFA has significantly improved understanding of nutritional impact on CVD, greater emphasis should be given for the whole foods containing SFA for achieving and maintaining cardiovascular health.

MCFAS with fatty acids moieties of 6-12 carbon atoms, found in coconut oil, are minor components of a normal diet. Besides natural sources, synthetic medium-chain triglyceride (MCT) oils are synthesized by hydrolysis of coconut or palm kernel oil, filtration of MCFAS, and subsequent re-esterification of glycerol with fatty acids. MCT oil were introduced into clinical nutrition in the 1950s as a special dietary energy source in various clinical settings, including pancreatic insufficiency, fat malabsorption, impaired lymphatic chylomicron transport and total parenteral nutrition due to its unique structural, absorption and metabolic characteristics (2, 43, 115). Ordinary dietary fats are mainly LCSFAS, which contain more than 12 carbon atoms, are found abundantly in dairy fats and animal products (lard, tallow, suet) (116). Compared to triglycerides containing LCSFAS, triglycerides containing MCFAS have unique physical and metabolic properties that demonstrate substantial metabolic differences. The medium chain triglyceride containing MCFAS are hydrolyzed by pancreatic lipase and are more rapidly absorbed in the duodenum compared to LCSFAS. Most absorbed MCFAS are transported predominantly by the portal vein to the liver, whereas LCSFAS are incorporated into chylomicron after absorption from the intestine for transport through the lymphatic system or peripheral circulation (2, 26). However, chronic MCFAS supplementation leads to an increase in the proportion of MCFAS in chylomicrons (117). In contrast to LCSFAS, MCFAS do not require carnitine to cross the double mitochondrial membrane of the hepatocyte and are rapidly oxidized. MCFAS oxidation is higher compared to LCSFAS. The increased production of acetyl CoA from MCFAS compared to LCSFAS leads to increased ketone body production. Contrary to LCSFAS, the cellular metabolism of MCFAS do not require binding fatty-acid binding protein, fatty acid transport protein, and/or fatty acid translocase (2, 26). MCFAS attenuate fat mass through down-regulation of expression of peroxisome proliferator activated receptor-g and adipogenic genes (118). It was, however, frequently demonstrated that MCFAS raise fasting cholesterol and triglyceride concentrations compared to soybean, corn, or olive oil (117, 119-121). However, PUFA themselves are hypocholesterolemic and hypotriglyceridemic compared to saturated other dietary fatty acids (122). MCFAS do not raise the triglyceride and chylomicron concentrations in the blood (123), these characteristics have led investigators to examine the differential effect of MCFAS with LCSFAS on fasting blood lipids (124), and the postprandial lipid response (125, 126).

1.7. IMPLICATIONS OF MCFAS FOR HEALTH:

1.7.1 Fasting plasma lipids

Blood lipids are secreted into the bloodstream from the intestinal mucosa as chylomicrons and from the liver as VLDL. In-vitro study have shown that MCFAS (caprylic acid) (127, 128), (capric and lauric acid) (97) stimulated apoB, triglyceride secretion less than LCSFAS (palmitic acid) and PUFA (oleic or linoleic acid) (129). Similarly, MCFAS (capric and lauric acid) reduced expression of apoB mRNA and intracellular accumulation of triglycerides. In addition, MCFAS also inhibited LCSFAS (palmitic acid) stimulated apoB secretion (127, 128). MCFAS reduce secretion of triglyceride when used in lower amounts; however, when MCFAS is consumed in excess of caloric needs, they could increase de novo lipogenesis. This, in turn, might increase secretion of triglyceride and could elevate fasting plasma triglyceride concentrations (130). However, several human studies have demonstrated that MCFAS elevated fasting plasma triglyceride and cholesterol levels compared with high PUFA diets (soybean or corn oil) (120, 131). There is enough evidence in the literature to demonstrate that n-6 PUFA lower blood triglyceride and LDL-C levels without affecting HDL-C levels compared to SFA (89). Based on this evidence, dietary guidelines recommend to lower SFA in the diet. However, lowering SFA in the diet may also reduce HDL-C levels; therefore, recent literature point to the importance of whole food containing different SFA chain lengths on blood lipid and lipoprotein levels. There are differential effects of MCFAS versus LCFAS diets on blood lipids and lipoprotein levels; MCFAS increased HDL-C and apo A-I levels compared to LCSFAS has been demonstrated for the first time and is explained in this thesis (Chapter 3).

1.7.2. Postprandial plasma lipids

Evidence regarding the role of meal fatty acid composition on postprandial lipemia is inconsistent. It has been shown that specific dietary fatty acids can modulate differently the peak plasma triglyceride concentration, the time of peak concentration and clearance rate of plasma triglyceride (132-136). However, the findings are inconsistent; few studies have demonstrated lower plasma triglyceride iAUC after ingestion of SFA rich meals compared with n-6PUFA and MUFA rich meals (134, 137); and another study

has demonstrated lower triglyceride iAUC after ingestion of n6PUFA compared to MUFA and SFA rich meals (138). The reason for this discrepancy, may be in part linked, to the fact that SFA varying in fatty acid chain lengths are metabolised differently and may modulate postprandial lipemia. The postprandial triglyceride response was generally lower with intake of MCFAS compared to MUFA and PUFA in animals (139). Several human studies comparing the effect of MCFAS with unsaturated fats for postprandial lipemia demonstrated lower postprandial triglyceride response following MCFAS (MCT oil) compared to corn oil. (140, 141) and soybean oil (120). This diminished postprandial triglyceride response compared to unsaturated fats is explained by the fact that MCFAS are not transported by chylomicrons, but instead travel as fatty acids via the portal vein to the liver. Thus, it appears that MCFAS attenuates the postprandial lipemic response to a fat meal compared to unsaturated fats. However, healthy male subjects fed MCT oil (MCFAS in excess 150% of their maintenance energy requirement) demonstrated higher postprandial triglyceridaemia (14). Thererfore, MCFAS when consumed in excess of energy, may result in increased hepatic fatty acid synthesis by providing increased amounts of acetyl CoA. Increased fatty acid synthesis may then result in increased hepatic triglyceride synthesis and elevated plasma triglyceride concentrations (131).

However, less is known about the effects MCFAS in the form of coconut oil on postprandial lipemia. Similarly, there is not much information regarding the differential effects of chain lengths of SFA food sources on postprandial lipemia. To our knowledge, we demonstrated for the first that coconut oil lowers postprandial lipemia compared to butter and lard in this thesis (chapter 4).

1.8. RESEARCH RATIONALE, HYPOTHESIS AND SIGNIFICANCE

1.8.1. Rationale

Dietary SFA consumption has been shown to increase LDL-C, and therefore has been linked with increased CVD risk. However, the results reported on the association between SFA and hyperlipidaemia as well as CVD risk are heterogenous, due to at least in part to the intrinsic limitations of clinical trials that have evaluated the relationship. This heterogenous results may also be attributed to the lack of knowledge and consideration

that all saturated fatty acids have the same effects on human health. In addition, the majority of studies reporting conflicting results have used individual SFA in their design, most likely because each type of saturated fatty acid has unique effects on cells and tissues. However, no individual SFA is ever consumed in isolation. Therefore, the examination of the major food sources of SFA was necessary to determine whether SFA chain lengths have differential impact on blood lipid and lipoprotein levels.

Dietary SCFA and MCFAS are not incorporated into chylomicrons triglycerides and transported through the lymph in humans, however are transported directly to the liver as free fatty acids. While long chain SFA are packaged into chylomicrons and enter lymphatic circulation. Although differences in the metabolic handling of SCFA/ MCFAS versus LCSFAS exist, little is known about the differential effect of SFA chain lengths on blood lipid levels. Thus, my research hypothesis being that SFA chain lengths (MCFAS and LCSFAS) consumed for long term (3-6 months) exert differential effect on blood lipids and lipoproteins (Chapter 3). A differential effect of SCFA was not studied in the meta analysis because they did not have sufficient research evidence to allow a systematic review and meta-analysis. In chapter 4, my research hypothesis being that SFA chain lengths (SCFA, MCFAS, LCSFAS) exert differential effects on blood lipid levels. A differential effect of SCFA was not apparent in this study (chapter 4). Another potential limitation of the study is that the amount of SCFA in the butter biscuit was not sufficient to counter-balance the negative effects of LCSFAS; therefore, the real effect of the SCFA could not be established.

1.8.1. Objective

The specific objectives of my candidacy were:

1.8.1.1 Objective 1: To systematically review and synthesize the evidence on the differential effects of long-term dietary supplementation rich in MCFAS or LCSFAS on blood lipd and lipoprotein levels.

1.8.1.2. Objective 2: To compare the efficacy of acute dietary supplementation with SCFA, MCFAS or LCSFAS on postprandial lipemia in healthy male and female subjects.

1.8.2. Significance

The effect of different chain-length fatty acids on metabolism is unclear in the literature. Not all saturated fats are the same, and the cardiovascular health of the population would benefit from greater understanding of the differential effects of healthy versus unhealthy saturated fats. The study hypothesises that short and medium chain saturated fat do not adversely affect lipid metabolism. This research may aid in the design of dietary strategies for cardiovascular risk reduction and contribute to revised dietary guidelines for healthy eating.

Chapter 2:

General Methods

2.1. Study 1: Systematic review and meta-analysis.

2.1.1 Search strategy, selection criteria and selection procedures

The review was performed using Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guideline. The inclusion criteria were clinical trials reporting the effect of MCFAS vs. LCSFAS dietary intervention on blood lipids (TG, TC, LDL-C, HDL-C). The following search terms were used and they were divided into three key area: (1) MCFAS: coconut oil, palm kernel oil, medium-chain triglycerides, medium-chain fatty acids, caproic acid, hexanoic acid, caprylic, octanoic acid, capric acid, decanoic acid, lauric acid, monolaurin, trilaurin; (2) LCSFAS: long-chain saturated fatty acid, tallow, beef fat, suet, lard, palm oil, butter, cheese, myristic acid, palmitic acid, stearic acid; (3) Intervention: randomized controlled trial, intervention studies, clinical trial(s), random, group, trial and; (4) Outcomes: Blood lipid, plasma lipid , serum lipid, LDL-C, HDL-C, TC/HDL cholesterol, LDL cholesterol/HDL, VLDL) cholesterol, triacylglycerol's, triglyceride, lipoprotein, dyslipidemia, hypercholesterolemia, lipid disorders, hyperlipidemia, hypolipidemia, hypertriglyceridemia. The search used in Scopus is included as an example (Appendix). A three-step search strategy was utilised in this review. Firstly, an initial limited search was done by using electronic databases (Medline, Scopus and CIHAHL) to identify any known systematic review on this topic. Studies were identified by a second search using all identified keywords and index terms through five electronic databases (EMBASE, MEDLINE, CINAHL, Cochrane and Scopus). Thirdly, the reference list of the retrieved publications were searched for additional studies. The search was restricted by the English language, and each database was searched from inception to April 2018. All studies identified during the database search were examined for relevance to the review based on the titles and abstract screening using eligibility criteria by two independent authors. Full article were retrieved for all studies that appeared to meet inclusion criteria and further assessment was done to determine eligibility. For any uncertainty in the decision making of selection, the third author was consulted until consensus was reached. Data extraction was conducted using a pretested data extraction spreadsheet.

2.1.2. Critical appraisal and data extraction and reporting

All papers selected for inclusion were assessed independently by two reviewers using the modified Cochrane Collaboration tool for cross-over studies to assess risk of bias. For any disagreement in the decision making, the third author was consulted until consensus was reached. Following a quality assessment, data was extracted from included studies by one reviewer using a pretested data extraction spreadsheet; this was cross-checked by a second reviewer for accuracy and completeness. Studies were described in terms of study identification (author, year, country), study design (cross-over or parallel, level of blinding), duration, sample size of each group, participant characteristics (age, gender, BMI, health status), intervention characteristics (MCFAS amounts and percent energy intake), comparator characteristics (LCSFAS amounts and percent energy intake) and blood lipid and lipoprotein outcomes (TG, TC, LDL-C, HDL-C, VLDL-C, apo A-I and apo B levels). The meta-analysis was performed using Review Manager Software 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration). The overall pooled effect was calculated using a random effects model described by Dersimonian and Laird (142) with inverse-variance (SE) weightings

2.2. Study 2: acute fat challenge study

2.2.1. Research study design

Acute trial investigating the effects of tests biscuits containing either butter fat (BB), coconut oil (CB) or lard (LB) on postprandial blood lipids (chapter 4) were monitored over a 24-hour period to assess the acute effects of supplementation with SCFA vs. MCFAS vs. LCSFAS. This was a randomized, cross-over, single-blinded design study with three experimental test days separated by at least a week of washout period. All subjects were randomly assigned to each of the interventional foods using a computer-based procedure. Test foods consisted of biscuits containing either 40g of butter fat (BB), 40g coconut oil (CB) or 40g lard (LB). The butter biscuits contained 61g carbohydrates, 7g protein and 37g fat (24g saturated, 7g monounsaturated, 1g polyunsaturated fat); the coconut biscuits contained 66g carbohydrates, 7g protein and 39g fat (35g saturated, 0.6g monounsaturated, 0.6g polyunsaturated fat) and the lard biscuits contained 66g carbohydrates, 7g protein and 39g fat (20g saturated, 14g monounsaturated, 1.5 g polyunsaturated fat). Butter contains SCFA (butyric acid) and long-chain SFA (myristic, palmitic and stearic acid). Coconut oil contains medium chain SFA (caprylic acid decanoic acid and lauric acid) and long chain SFA (myristic and palmitic acid). Lard contains long chain SFA (palmitic acid, stearic acid and myristic acid) (143, 144).

2.2.2. Participants

Participants were recruited from the student population of the University of Newcastle and general community of Newcastle, Australia via advertisements on the noticeboards at the University of Newcastle and through the Hunter Medical Research Institute (HMRI) Volunteer Register. Recruitment flyers were also distributed via department and faculty email list. All participants attended the research unit on 3 separate occasions to consume different test biscuits on each occasion with a minimum one-week washout period in between. Participants underwent study procedures at the Neutraceuticals Research Group Clinic rooms, University of Newcastle, NSW, Australia.

2.2.3. Inclusion/Exclusion criteria

Participants that were recruited for the study were healthy male or female adults 18 years of age or older with body mass index (BMI) between 18 and 25Kg/m^2 at intitial assessment that were able to participate and attend the research premises at the University of Newcastle, Australia. Participants were excluded if they were using lipid-lowering drugs (e.g. statins); had consumed fish oil supplements regularly within the past month; had regular consumption of two or more fish meals a week over the past month; had any history of congestive heart failure, stroke, myocardial infarction, coronary artery bypass graft, or established atherosclerotic disease; a history of diabetes, gastrointestinal or liver disease; were current smokers; or were pregnant or breastfeeding. All participants gave written informed consent.

2.2.4. Ethics and trial registrations

All participants provided written informed consent according to protocol guidelines approved by institutional regulations concerning the ethical use of human volunteers. The study was approved by the University of Newcastle Human Research Ethics Committee (protocol H-2016-0429) and registered with the Australia New Zealand Trial registry as ACTRN12617000903381. The studies were conducted in accordance with The Declaration of Helsinki.

2.2.5. Anthropometric measurements

All anthropometric measurements were performed in the morning, after an overnight fast with the participant in bare foot and light clothes. Participant's standing height was recorded to the nearest 0.1 cm using a using a wall-mounted stadiometer. Body weight and composition were measured using an InBody 230 (Biospace Co., Ltd. Seoul, Korea). Blood pressure was measured using an automated sphygmomanometer (Pulsecor Cardioscope). Body mass index (BMI) was calculated as the weight in Kilograms (kg) divided by the square of the height in meters (m) to the nearest 0.1 (kg/m^2). Waist circumference was measured at the mid-point between the lowest rib and iliac crest. Hip measurement was taken at the fullest point of the hip, as observed from the side. The same

research personnel measures anthropometry using the same equipment at all the visits to minimize variation in the results.

The InBody 230 machine uses direct segmental multi-frequency bio-electrical impedance analysis to estimate body composition, and in particularly body fat in the trunk, arms and legs. The subject stands on the base of the machine making contact of feet soles and hand with electrodes after the areas were cleaned with alcohol wipes. There are eight contact points in total, two on the side of eacvh hand that grip the handles and two on each foot.

2.2.6. Medical Questionnaire

A general medical questionnaire was collected from all subjects upon entry into the study (Appendix 10). The medical questionnaire included question regarding the participants past medical history, current medical conditions and current prescribed medications being taken.

2.2.7. Physical activity assessment

The International Physical Activity Questionnaire (IPAQ, https://sites.google.com/site/theipaq/questionnaire_links) long format was used to assess the level of physical activity during the study period (Appendix 4). Participants completed the questionnaire at their first visits to the Nutraceutical Research Clinical facilities. All participants were encouraged to maintain their physical activity status during the study period to avoid changing other variables which could influence postprandial blood lipids.

2.2.8. Blood pressure

Blood pressure was measured using an automated sphygmomanometer (Pulsecor Cardioscope). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken in the supported left arm after participants were seated for at least 5 minutes. Cuff size adjustment was based on arm circumference. SBP and DBP were based on the average of two separated measurements taken atleast 5 minutes apart by the investigating researcher.

2.2.9. Dietary recall (24-hour)

The participants got instructions on how to record their food consumption information in a 24-hour food diary. At baseline, the previous day's food intake was collected using a 24-hour food recall (Appendix 9). Everything eaten and drunk during the period was reported using household measures (cup, spoon, etc.), weight and food whole or part (e.g. one carrot). The 24-hour food recalls were entered into computer based nutrient analysis (FoodWorks 8.0.3551, Xyris software, Australia Private Ltd) to obtain estimated energy intake.

2.3.0. Sample size

The sample size was calculated by using Paired t-test (Formula 1). This study was designed to evaluate the differential postprandial effects on blood lipid levels of short, medium and long chain saturated fatty acids, and therefore TG levels was used as endpoint to calculate sample size. The sample size calculation is based on an expected 130% (88.8) difference in incremental area under the curve (iAUC) between test foods for plasma triglycerides with level of significance 0.05 and 80% power. Using a standard deviation of 114 (145) in iAUC and 10% dropout rate, we recruited 15 subjects.

$$(1) \qquad n = \left(\frac{(Z_\alpha + Z_\beta)\sigma_d}{\Delta} \right)^2$$

Where n is the number of subjects, Z_β is the value of standard normal distribution cutting off probability β, Z_α is the value of the standard normal distribution cutting off probability α, σ_d is the standard deviation of the within pair differences and Δ is the difference to be detected.

Chapter 3:

Differential effects of medium and long-chain saturated fatty acids on blood lipid profile: a systematic review and meta-analysis

The content described by this chapter has been submitted as the following manuscript:

Panth N, Abbott KAA, Dias CB and Garg ML. Differential effects of medium and long-chain saturated fatty acids on blood lipid profile: a systematic review and meta-analysis. The American Journal of Clinical Nutrition, 2018.108(4): 675-687. DOI: 10.1093/ajcn/nqy167

Statement of authorship in appendix 1

Research in human models suggest that MCFAS consumption does not affect blood lipid levels adversely. Therefore, our aim in Chapter 3 was to systematically evaluate the literature on the differential effect of chain length of SFA (MCFAS and LCSFAS) on blood lipid levels.

Abstract

Background: Medium-chain saturated fatty acids (MCFAS) may affect circulating lipids and lipoproteins differently than long-chain saturated fatty acids (LCSFAS), however results from human intervention trials have been equivocal**.**

Objective: The aim was to determine whether MCFAS and LCSFAS have differential impact on blood lipids and lipoproteins.

Design: Five databases were searched (EMBASE, MEDLINE, CINAHL, Cochrane and Scopus) until April 2018 and published clinical trials investigating the differential effects of dietary MCFAS and LCSFAS on blood lipids were included. Searches were limited to the English language and to studies with adults aged >18 y. Where possible, studies were pooled for meta-analysis using RevMan® 5.2. The principle summary measure was the mean difference between groups calculated using the random effects model.

Results: Eleven eligible cross-over and a one parallel trial were identified with a total of 299 participants [weighted means ± SDs age: 38 ± 3 y; weighted mean ± SDs body mass index (BMI): 24 ± 2 kg/m^2]. All studies were pooled for the meta-analysis. Diets enriched with MCFAS led to significantly higher high-density lipoprotein cholesterol (HDL-C) levels compared to LCSFAS (0.11 mmol/L; 95% CI: 0.07, 0.15) with no effect on triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels. Consumption of diets rich in MCFAS significantly increased apolipoprotein A-I (apo A-I) level compared to LCSFAS (0.08 g/L; 95% CI: 0.02, 0.14). There was no evidence of statistical heterogeneity for HDL-C, apo A-I, and TG levels, however significant heterogeneity was observed for the TC (I^2 = 49%) and LDL-C analysis (I^2 = 58%)

Conclusion: The findings of this research demonstrate a differential effect of MCFAS and LCSFAS on HDL-C levels. Further investigations are warranted to elucidate the mechanism by which the lipid profile is altered.

3.1. Introduction

Dyslipidemia, characterised by raised triglyceride (TG) levels, elevated low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) levels, is associated with adverse cardiovascular outcome (146). Dietary fats modulate circulating lipid levels and are associated with CVD risk (147). Cis isomers of monounsaturated fatty acids (MUFA) lower LDL-C and increase HDL-C levels (148). Polyunsaturated fatty acids (PUFA) of the omega-6 (n-6) family including linoleic acid, which accounts for more than 90% of daily PUFA intake, have been shown to lower total cholesterol (TC) and LDL-C (149, 150) levels. Current dietary guidelines encourage low consumption of saturated fatty acids (SFA) to avoid an increase in LDL-C levels (151). However, the blanket recommendation of reducing SFA for CVD prevention has recently been called into question (143). Reducing SFA intake also lowers HDL-C (104). Since HDL-C levels is inversely associated with CVD risk (152), the effect of reducing SFA consumption on overall CVD risk remains unclear.

In addition to the degree of saturation, there is evidence to suggest that the chain length of SFA is a major determinant of their lipid-modulating affect (116). Short-chain (SCFA 2-4 carbon atoms) are usually found in low proportions in the diet (144) and are produced by gut-microbiota fermentation of dietary fibres, with small amounts also found in dairy and fermented food products such as vinegar and fermented pickles (153). Medium chain (MCFAS 6-12 carbon atoms) present naturally in coconut and palm kernel oils or medium chain triglyceride (MCT) oils produced by re-esterification process (154) and long-chain (LCSFAS >12 carbon atoms) SFA found abundantly in dairy fat, tallow, lard and palm oil (155-157).

Preclinical evidence suggests that MCFAS decrease TG and increase HDL-C in comparison to LCSFAS (158, 159). These effects may be attributable to their unique metabolic pathway, whereby MCFAS are directly transported to the liver via portal circulation, and demonstrate higher rates of mitochondrial oxidation (116). It has been proposed that MCFAS may be beneficial for a variety of conditions, including hyperlipidemia (154), obesity (116) and memory impairment for example Alzheimer's disease (160). LCSFAS follow a complicated pathway of incorporation into chylomicrons and reaching the bloodstream via the lymphatic circulation. Together with chylomicrons, hepatic derived very low-density lipoprotein (VLDL) comprise the triglyceride-rich lipoproteins.

Several human intervention studies have investigated differential effects of MCFAS and LCSFAS on blood lipid profiles with mixed results however, to date, no studies have employed a systematic review process or performed a meta-analysis to assess the differential effects of MCFAS and LCSFAS on blood lipid and lipoprotein levels. Therefore, the aim of this review was to synthesize the evidence on the differential effects of MCFAS and LCSFAS on blood lipid and lipoprotein levels.

3.2. Methods

This systematic review and meta-analysis follows the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement (PRISMA) (161). The protocol was published in the PROSPERO database (www.crd.york.ac.uk/PROSPERO), under registration number CRD42017078277.

3.2.1. Search strategy

Studies were identified through five electronic databases (EMBASE, MEDLINE, CINAHL, Cochrane and Scopus). The following Medical Subject Headings (MeSH), words and their combinations were searched: coconut oil OR palm kernel oil OR medium-chain triglycerides OR medium-chain fatty acids OR caproic acid OR hexanoic acid OR caprylic OR octanoic acid OR capric acid OR decanoic acid OR lauric acid OR monolaurin OR trilaurin; AND long-chain saturated fatty acid OR tallow OR beef fat OR suet OR lard OR palm oil OR butter OR cheese OR myristic acid OR palmitic acid OR stearic acid; AND randomized controlled trial OR intervention studies OR clinical trial(s) OR random* OR group* OR trial*; AND blood lipid OR plasma lipid OR serum lipid OR low-density lipoprotein (LDL) cholesterol OR high-density lipoprotein (HDL) cholesterol OR cholesterol OR TC/HDL cholesterol OR LDL cholesterol/HDL OR very low-density lipoprotein (VLDL) cholesterol OR triacylglycerol's OR triglyceride OR lipoprotein OR Dyslipidemia OR hypercholesterolemia OR lipid disorders OR hyperlipidemia OR hypolipidemia OR hypertriglyceridemia. To optimise publication retrieval, the internationally broad MeSH terms and spelling were used. References of the retrieved publications were examined to identify other potential papers for inclusion. Limits included publications prior to 1st April 2018 and English language. Primary outcomes collected were TG, LDL-C, TC and HDL-C levels and secondary outcomes included VLDL-C, Apolipoprotein A-I (apo A-I), Apolipoprotein B (apo B) levels.

3.2.2. Eligibility criteria

Publications were included if they had a dietary intervention of any duration that compared a MCFAS with a LCSFAS and they reported blood lipids (TG, TC, LDL-C, HDL-C) as an outcome. Postprandial studies, observational studies or those that did not report blood lipids (TG, TC, LDL-C, HDL-C) as an outcome measure or those that did not compare MCFAS with LCSFAS were excluded from this review. Studies were also excluded if they were conducted in populations on enteral or parenteral nutrition.

3.2.3. Selection process and quality assessment

The titles and abstracts of all articles were screened by two authors (NP, KAA) for the first selection process using the eligibility criteria. If there was insufficient information in the abstract to warrant exclusion of an article, the full text of the article was retrieved to determine eligibility. The full text of all publications that appeared to meet the eligibility screening process were retrieved and a second selection assessment was undertaken. Any uncertainty in the assessment and/or the decision-making of selection was resolved after discussion with another independent research investigator (MLG). Two independent research investigators (NP, KAA) assessed the methodological quality of the selected full texts using the modified Cochrane Collaboration tool for cross-over studies to assess risk of bias (162). The Cochrane tool aided in critically appraising the quality constructs of each publication as well as to determine the relevance and validity of the selected publication. Biases are assessed as a judgment (high, low, or unclear) for individual elements from six domains (sequence generation, carry-over effects, allocation concealment, blinding of the participants and study personnel, handling of missing data (intention-to-treat or per protocol analysis), and selective outcome reporting). Then, each trial received an overall classification of risk of bias. Based on empirical evidence, carry-over effects and sequence generation were considered the main categories that could bias the overall results of the analysis (163).

3.2.4. Data extraction

Relevant data from included publications was extracted by one author (NP) using a pretested data extraction spreadsheet; this was checked by a second reviewer (KAA) for accuracy and completeness. The following data were collected: study identification (author, year, country), study design (cross-over or parallel, level of blinding), duration,

sample size of each group, participant characteristics (age, gender, BMI, health status), intervention characteristics (MCFAS amounts and percent energy intake), comparator characteristics (LCSFAS amounts and percent energy intake) and blood lipid and lipoprotein outcomes (TG, TC, LDL-C, HDL-C, VLDL-C, apo A-I and apo B levels). When the MCFAS amounts were not reported, they were derived from the given fatty acids composition of the diet (percent energy intake) and reported mean energy intake of the MCFAS groups. Mean and variance measures such as standard deviations (SDs) and standard error of the means (SEMs) were collected where possible. Any studies that reported SEMs had the SEMs converted to SDs with the use of the following formula: SDs = SEMs $\times\sqrt{n}$ (164). MCFAS amounts were categorised into as low (14-18 g/day), moderate (20-32 g/day) and high (59-108 g/day) amounts. Publications that reported TG, TC, LDL-C, HDL-C values in mg/dL were converted to mmol/L using the standardised conversion method (165). Studies that provided apo A-I and apo B values in mg/dl or mg/L were converted to g/L.

3.2.5. Statistical analysis:

Data for each outcome were pooled for the meta-analysis if the outcome was reported in more than 3 studies, and if the group means and either SDs or SEMs were reported for both the MCFAS and the LCSFAS groups. Crossover trials reported group differences post-intervention and a parallel trial reported pre-intervention and post-intervention outcomes. Therefore, for cross-over studies, the primary outcome measure was the mean difference (MCFAS-LCSFAS) of the post-intervention values. Similarly, for a parallel design study, the principal outcome measure was the mean difference (MCFAS-LCSFAS) of the change from baseline values (post-intervention minus baseline values) between groups. Combining both the post-intervention and change from baseline values in one meta-analysis is an accepted method of combining cross-over and parallel design studies in a meta-analysis as described by the Cochrane Collaboration (166). The overall pooled effect was calculated using a random effects model described by Dersimonian and Laird (142) with inverse-variance (SE) weighting. Heterogeneity among the studies was tested using the I^2 statistics. I^2 values of approximately 25%, 50%, and 75% are considered to demonstrate low, moderate, and high levels of heterogeneity, respectively (167). For each outcome measure, a sensitivity analysis was conducted to examine whether a particular study elicited any undue influence on the overall outcome. This was

conducted by exclusion of each study one at a time and recalculation of the effect size. To investigate the effect of study quality, additional sensitivity analysis on main outcomes were conducted including trials considered to have a low risk of bias according to the Cochrane tool. Publication bias was tested by visual inspection of funnel plot and formally tested by Egger's regression test (168).

A potential linear dose-response, and non-linear dose-response relation, between MCFAS dose (g/d) and change in HDL-C concentration was examined by meta-regression analysis and fractional polynomial regression analysis respectively. Both the regression analysis were carried out using Stata IC 14 (StataCorp LP). In these dose-response models, the coefficient for the MCFAS dose was an estimate of the dose-response relation between MCFAS dose and change in HDL-C concentration.

A subgroup analysis of the amounts of MCFAS (14-18 g/day: low amounts; 20-32 g/day: moderate amounts, and 50-108 g/day: high amounts), the study design (with and without washout periods) and the source of MCFAS (naturally occurring and manufactured MCFAS) were performed. Review Manager Software 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration) was used for the meta-analysis. All data are reported as means ± SDs. *P*-values <0.05 were considered statistically significant.

3.3. Results

3.3.1. Study and participant characteristics

An overview of search results is presented in the flow diagram in **Figure 3.1**. After excluding duplicates, the initial search identified 214 papers for screening. A total of 11 cross-over and a one parallel design studies, including n = 299 participants, met the inclusion criteria. Nine studies gave interventions in a random order, whilst one was sequential and two were given in a non-random order. A summary of study characteristics is shown in **Table 3.1**. Of 12 studies, six included only male participants, one included women exclusively, and the remaining five had males and females pooled together. Publications included Americans (n = 4), Asians (n = 2), and Europeans (n = 6). Majority of the studies (n = 10) were conducted in healthy populations while a small number (n = 2) in hypercholesterolemic (plasma cholesterol >5.5 mmol/L) individuals. Studies were open label (n = 10, where both study participants and investigators are aware of group assignment), single blinded (n = 1, where study participants are unaware of group assignment), and double blinded (n = 1, where both study participants and investigators

are unaware of group assignment). Trials ranged from three to six weeks (median = 4 weeks) in duration. Cross-over studies used a washout period of one week to two months (n = 8, where participants were taken off the study intervention or comparator in order to eliminate their short-term carry over effects) and three studies had no washout period. Ten studies reported body mass index (BMI). The weighted BMI across studies was 23.8 ± 2.28 kg/m^2, range 21-29 kg/m^2. Eight studies reported BMI in the healthy category (BMI 18.5-24.9 kg/m^2) and two studies reported BMI in the overweight category (BMI: 25-29.9 kg/m^2). Ten studies reported a mean age of participants. The weighted mean age across studies was 38 ± 3 y, range 21-66 y). MCFAS amount ranged from 14.2-108 g/day. Interventions included naturally occurring sources of MCFAS (coconut oil, n = 6; lauric acid oil blend, n = 1), and lauric/myristic acid oil blend (n = 3) and manufactured (re-esterified) MCFAS (trilaurin oil, n = 1; MCT oil, n = 1). Comparators included palm oil (n = 5), beef fat (n = 1), butter (n = 3), and palmitic acid oil blend (n = 3).

Figure 3.1.

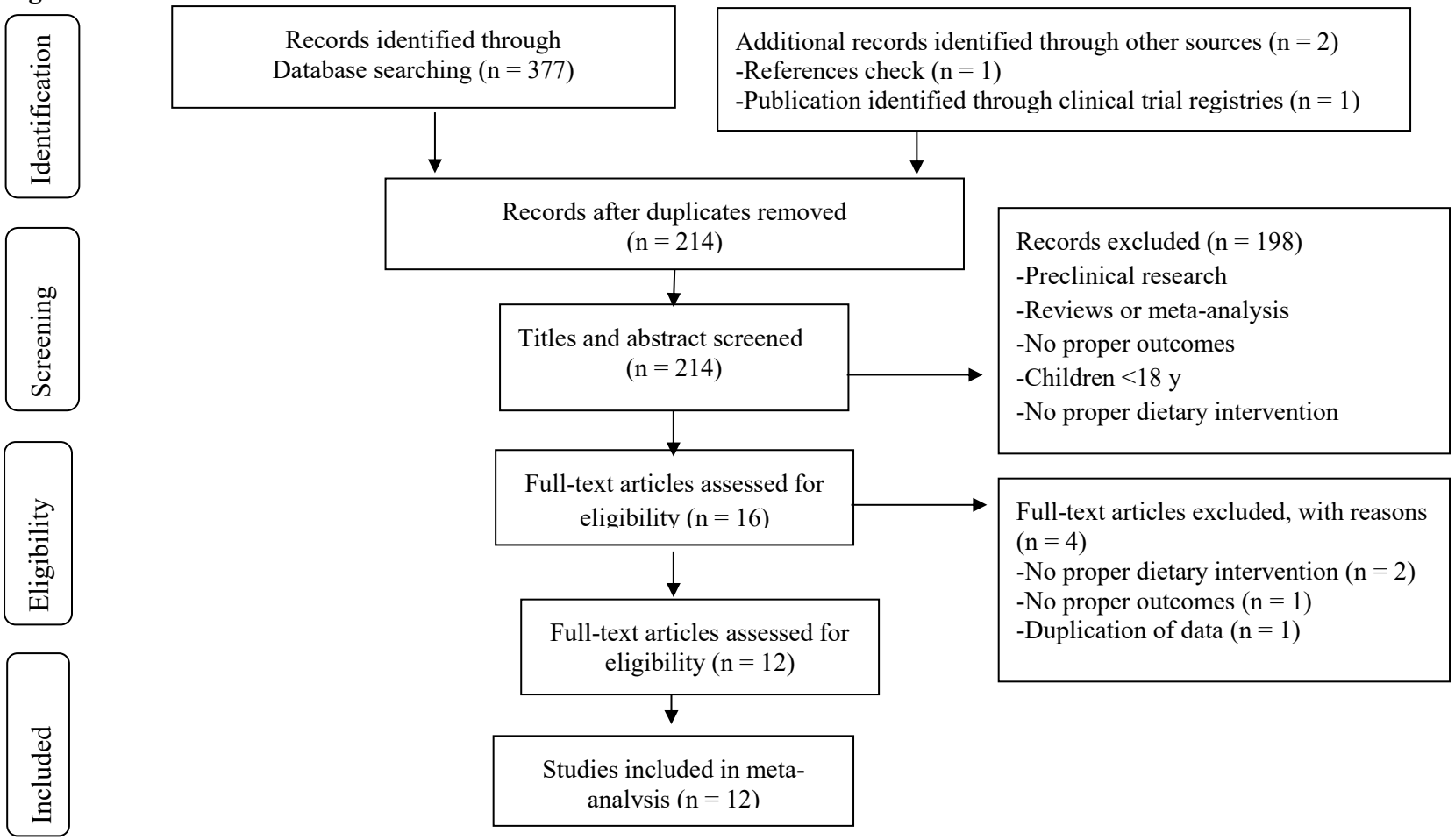


Figure 3.1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram depicting overview of study-selection process

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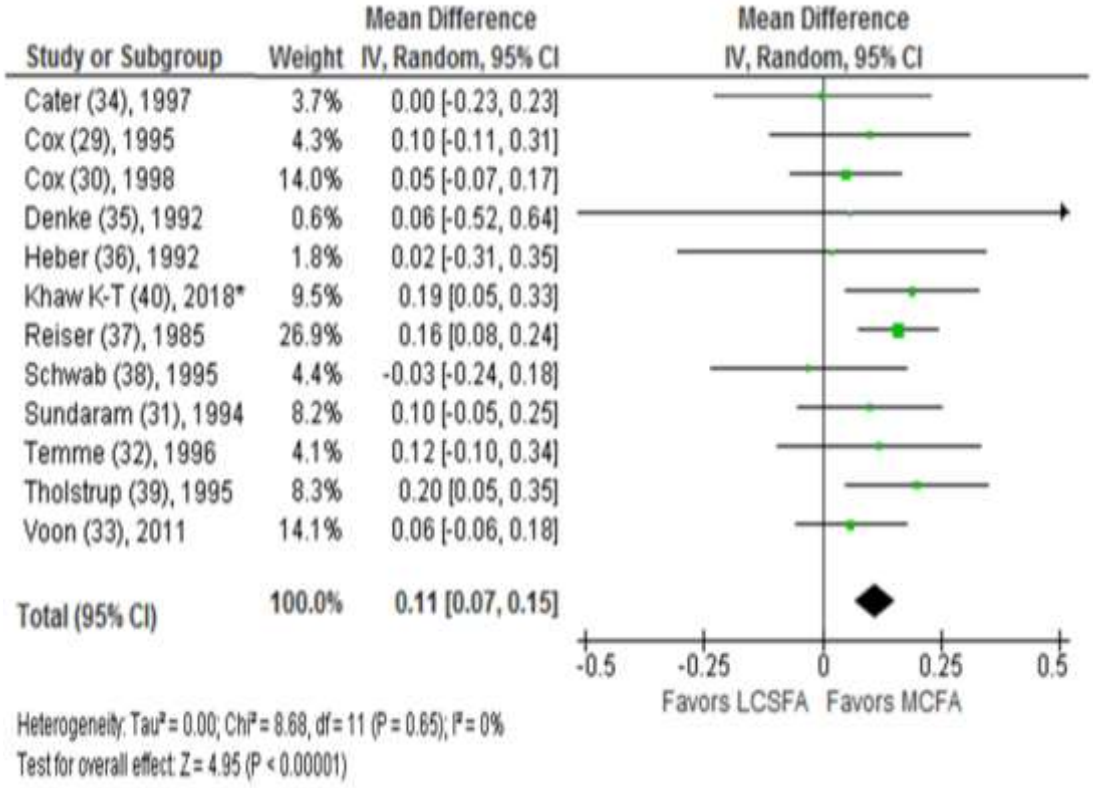
3.3.2. Overall effects of the MCFAS diet compared with those of the LCSFAS diet on lipid profile and study heterogeneity

All included studies were pooled for meta-analysis. **Figure 3.2** provides a summary of pooled estimates of HDL-C and apo A-I levels. The MCFAS diet led to significantly higher fasting HDL-C levels than LCSFAS diet (0.11 mmol/L; 95% CI: 0.07, 0.15) and there was a significant increase in fasting apo A-I compared to LCSFAS (0.08 g/L; 95% CI: 0.02, 0.14). **Figure 3.3** demonstrates a summary of pooled estimates of TG and TC levels; **Figure 3.4** shows a summary of LDL-C, VLDL-C and apo B levels. There were no significant differences in TC, LDL-C, TG, VLDL-C, and apo B levels between a MCFAS and LCSFAS diets. The overall pooled mean difference (95% CI) between a MCFAS and LCSFAS diet for TG, LDL-C, and TC were -0.03 mmol/L (95% CI: -0.09, 0.04), -0.00 mmol/L (95% CI: -0.18, 0.18), and 0.11 mmol/L (95% CI: -0.06, 0.28) respectively. Six studies reported VLDL-C, apo A-1 levels, and five studies reported apo B levels, and these were pooled. The overall mean difference (95% CI) between a MCFAS and LCSFAS diet for VLDL-C and apo B levels were 0.06 mmol/L (95% CI: -0.02, 0.14) and -0.00 mmol/L (95% CI: -0.05, 0.05) respectively. Low to moderate between study heterogeneity was evident for the TG, HDL-C, VLDL, apo A-I, and apo B levels (I^2 = 0%; I^2 = 0%; I^2 = 0%; I^2 = 10%, and I^2 = 2%; respectively). However, there was evidence of significant heterogeneity for TC (I^2 = 49%) and LDL-C levels (I^2 = 58%).

In the sensitivity analysis, we found no relevant differences compared to original analysis in most of our results when we limited the analysis to studies with low risk of bias. In addition, no evidence of significant heterogeneity was observed between studies, except for TC (I^2 = 52%) and LDL-C levels (I^2 = 65%) (**Table 3.3**).

Figure 3.2.

A


Study or Subgroup
Weight
Mean Difference
IV, Random, 95% CI
Mean Difference
IV, Random, 95% CI
Cater (34), 1997 3.7% 0.00 [-0.23, 0.23]
Cox (29), 1995 4.3% 0.10 [-0.11, 0.31]
Cox (30), 1998 14.0% 0.05 [-0.07, 0.17]
Denke (35), 1992 0.6% 0.06 [-0.52, 0.64]
Heber (36), 1992 1.8% 0.02 [-0.31, 0.35]
Khaw K-T (40), 2018* 9.5% 0.19 [0.05, 0.33]
Reiser (37), 1985 26.9% 0.16 [0.08, 0.24]
Schwab (38), 1995 4.4% -0.03 [-0.24, 0.18]
Sundaram (31), 1994 8.2% 0.10 [-0.05, 0.25]
Temme (32), 1996 4.1% 0.12 [-0.10, 0.34]
Tholstrup (39), 1995 8.3% 0.20 [0.05, 0.35]
Voon (33), 2011 14.1% 0.06 [-0.06, 0.18]
Total (95% CI) 100.0% 0.11 [0.07, 0.15]
-0.5
-0.25
0
0.25
0.5
Favors LCSFA
Favors MCFA
Heterogeneity: Tau² = 0.00; Chi² = 8.68, df = 11 (P = 0.65); I² = 0%
Test for overall effect: Z = 4.95 (P < 0.00001)

B

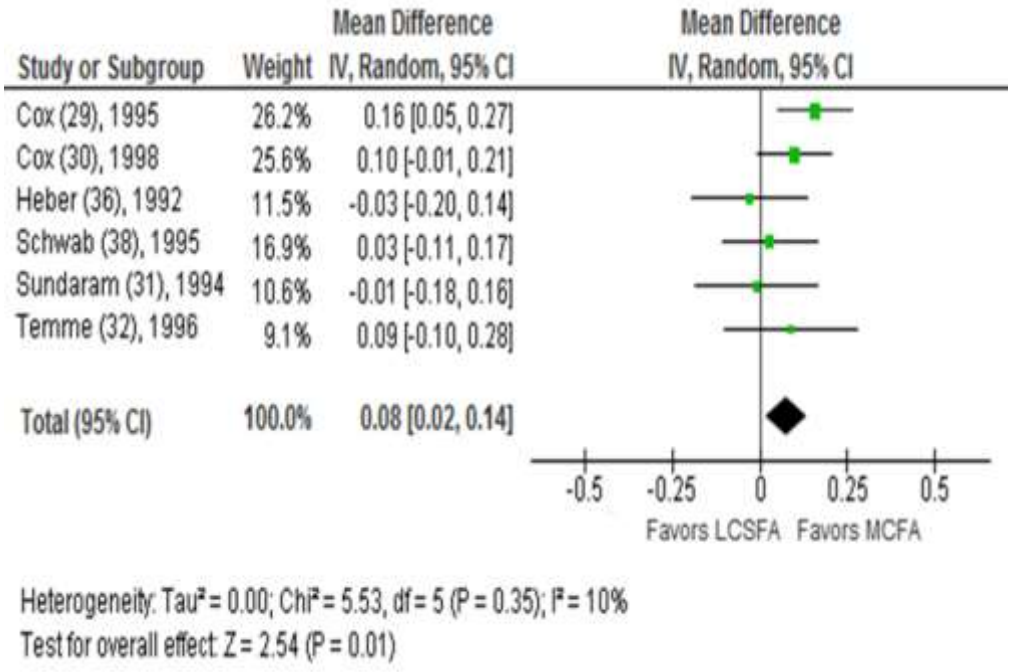
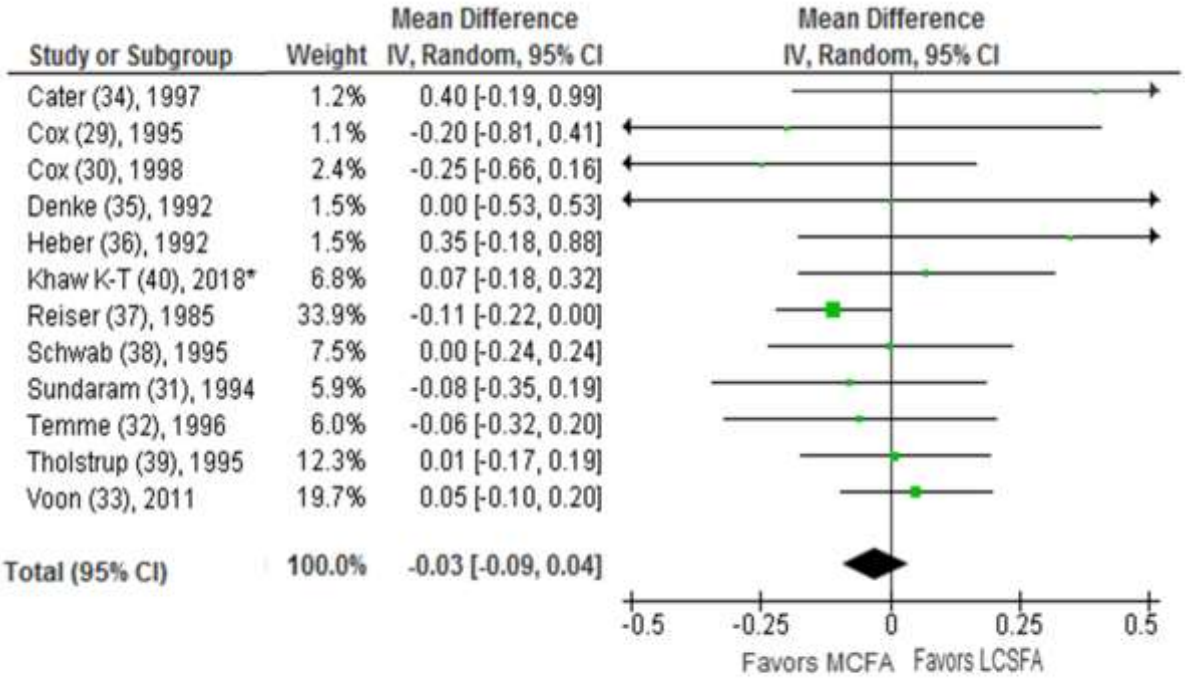


Figure 3.2. Meta-analysis of the effect of MCFAS intervention on measures of HDL-C (A) and apo A-I (B) levels. The summary measure for cross-over trial is the mean difference (mmol/L) and 95% CI between groups (MCFAS compared with LCSFAS). *The principal summary measure for the one parallel trial is the mean difference of the change from baseline (post intervention minus baseline) between groups (MCFAS compared with LCSFAS). The meta-analysis was performed using generic inverse variance weighting methods with a Dersimonian and Laird random effects model. The green squares represent the point estimates, with the size of the square demonstrating the weight of each trial included in the meta-analysis. The black vertical line represents the point of no effect. Black diamonds represent the pooled estimate effects of MCFAS intervention on circulating blood lipids and lipoproteins.
HDL-C, High-density lipoprotein cholesterol; apo A-I, Apolipoprotein A-I; MCFAS, Medium-chain saturated fatty acids; LCSFAS, Long-chain saturated fatty acids; CI, Confidence interval.

Figure 3.3

A



Heterogeneity: Tau² = 0.00; Chi² = 9.66, df = 11 (P = 0.56); I² = 0%
Test for overall effect: Z = 0.85 (P = 0.40)

B

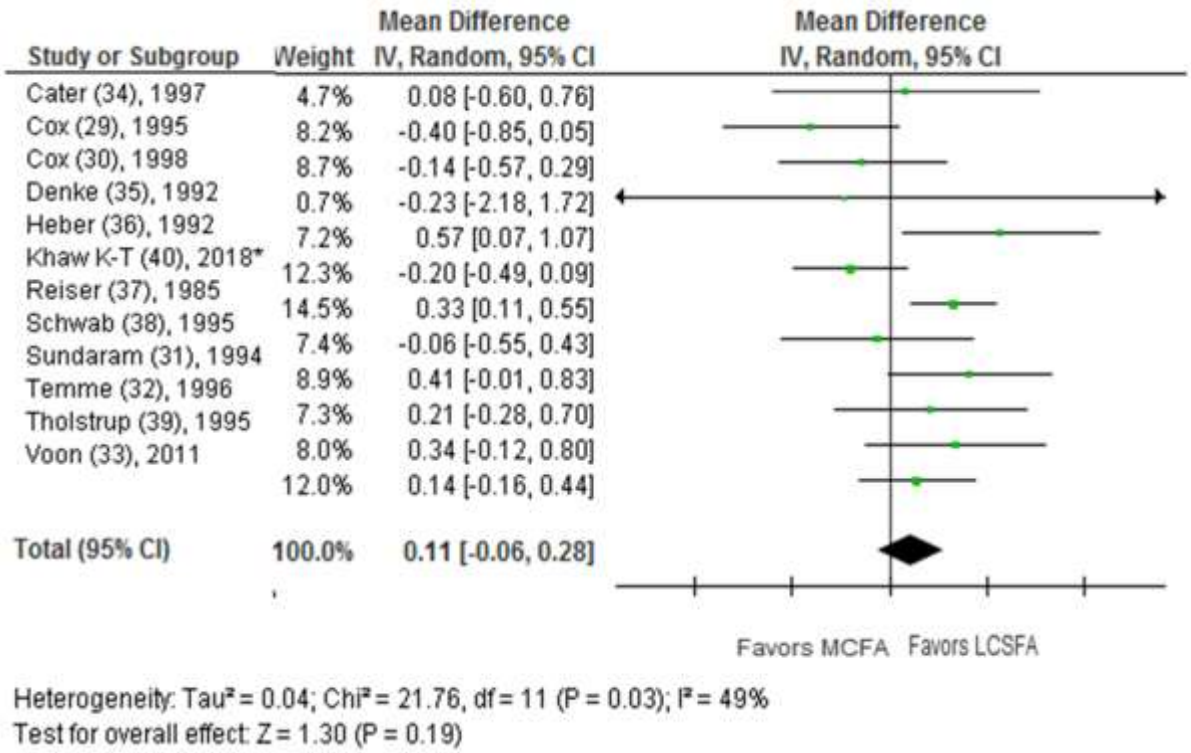
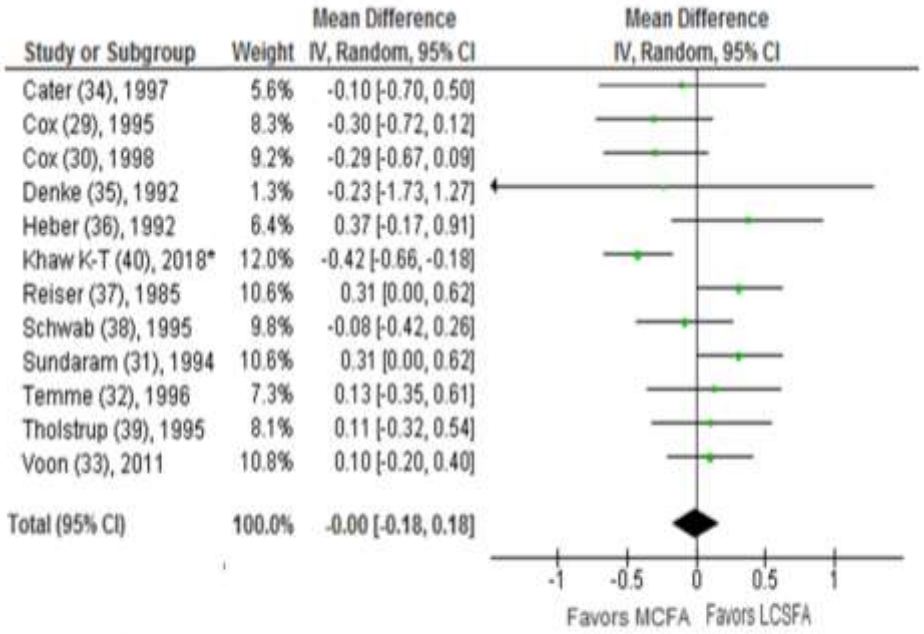


Figure 3.3. Meta-analysis of the effect of MCFAS intervention on measures of TG (A) and TC (B) levels. The summary measure for cross-over trial is the mean difference (mmol/L) and 95% CI between groups (MCFAS compared with LCSFAS). *The principal summary measure for the one parallel trial is the mean difference of the change from baseline (post intervention minus baseline) between groups (MCFAS compared with LCSFAS). The meta-analysis was performed using generic inverse variance weighting methods with a Dersimonian and Laird random effects model. The green squares represent the point estimates, with the size of the square demonstrating the weight of each trial included in the meta-analysis. The black vertical line represents the point of no effect. Black diamonds represent the pooled estimate effects of MCFAS intervention on circulating blood lipids and lipoproteins.

TG, Triglyceride; TC, Total cholesterol; MCFAS, Medium-chain saturated fatty acids; LCSFAS, Long-chain saturated fatty acids; CI, Confidence interval

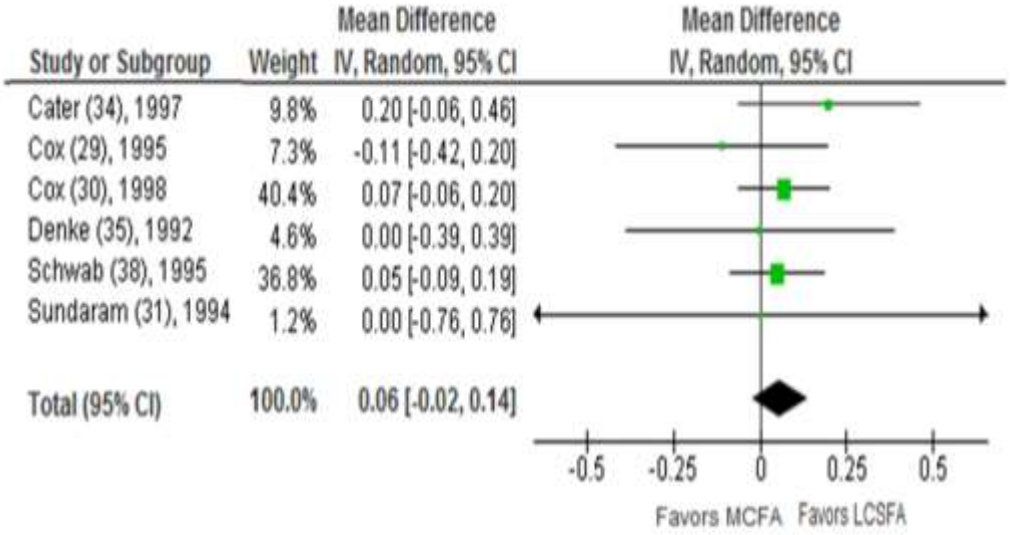
Figure 3.4.

A



Heterogeneity: $Tau^2 = 0.05$; $Chi^2 = 26.35$, df = 11 (P = 0.006); $I^2 = 58\%$
Test for overall effect: Z = 0.00 (P = 1.00)

B



Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 2.43$, df = 5 (P = 0.79); $I^2 = 0\%$
Test for overall effect: Z = 1.39 (P = 0.17)

C

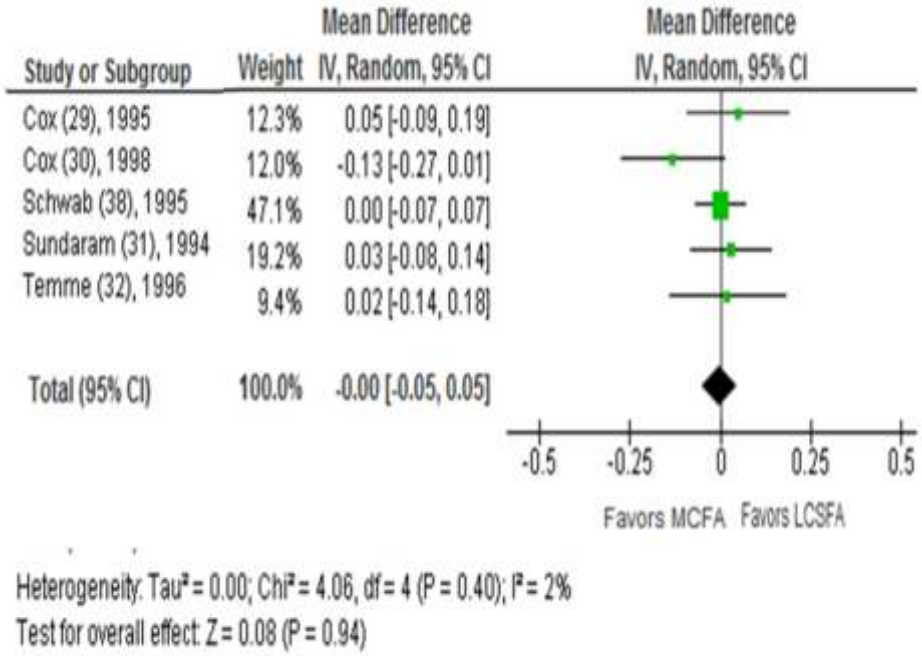


Figure 3.4. Meta-analysis of the effect of MCFAS intervention on LDL-C (A), VLDL-C (B), and apo B (C) levels. The summary measure for cross-over trial is the mean difference (mmol/L) and 95% CI between groups (MCFAS compared with LCSFAS). *The principal summary measure for the one parallel trial is the mean difference of the change from baseline (post intervention minus baseline) between groups (MCFAS compared with LCSFAS). The meta-analysis was performed using generic inverse variance weighting methods with a Dersimonian and Laird random effects model. The green squares represent the point estimates, with the size of the square demonstrating the weight of each trial included in the meta-analysis. The black vertical line represents the point of no effect. Black diamonds represent the pooled estimate effects of MCFAS intervention on circulating blood lipids and lipoproteins.

LDL-C, Low-density lipoprotein cholesterol; VLDL-C, Very low-density lipoprotein cholesterol; apo B, Apolipoprotein B; MCFAS, Medium-chain saturated fatty acids; LCSFAS, Long-chain saturated fatty acids; CI, Confidence interval

3.3.3. Subgroup analysis:

Subgroup analysis were conducted with the data segregated by the amounts of MCFAS (14-18 g/day, low amounts; 20-32 g/day, moderate amounts and 50-108 g/day, high amounts) and the study design (with and without washout period) (Table 3.3). The results for TG and LDL-C levels from all subgroup analysis were consistent with the findings of the combined analysis. Subgroup analysis based on the amounts of MCFAS revealed that high amounts of MCFAS significantly increased TC levels with no evidence of heterogeneity (I^2 = 0%), and both moderate and high amounts of MCFAS increased HDL-C levels without heterogeneity (I^2 = 0%). In the meta-regression, we did not observe a significant (P = 0.86) linear dose response between MCFAS and change in HDL-C concentration (Data not shown). However, In the non-linear dose-response analysis, significant dose-response relationship up to approximately 50 g/day MCFAS intake was observed (β=0.087; 95% CI: 0.007, 0.168), where it plateaued and no further response was seen (p=0.114) (**Figure 3.5**).

Subgroup analysis according to the study design, MCFAS significantly increased HDL-C and TC levels only in studies including a washout period. There was evidence of significant heterogeneity for TC levels (I^2 = 62%), however not for HDL-C levels (I^2 = 0%). Two studies used re-esterified MCFAS; subgroup analysis excluding these two studies from analysis showed that the effect size of HDL-C was similar to the original analysis. However, as only two studies used re-esterified MCFAS in the intervention, the data could not be pooled from two studies for the meta-analysis.

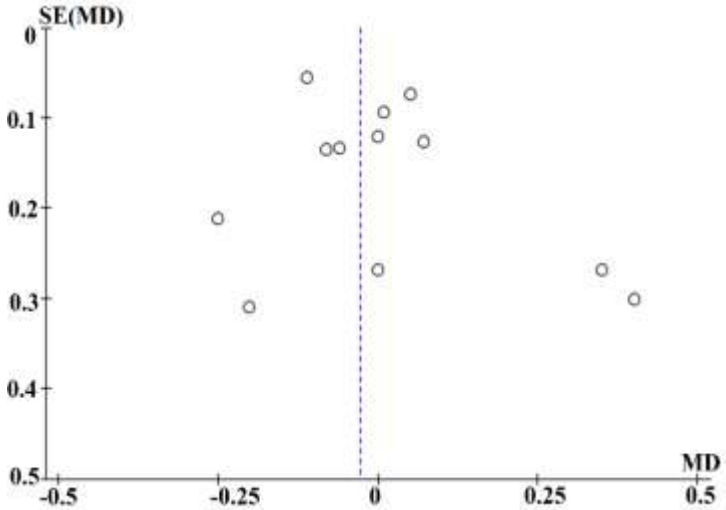
Potential sources of inter-study heterogeneity for TC and LDL-C levels were also investigated using additional subgroup analysis. We classified studies according to the participant characteristics (healthy and hyperlipidemia), duration of the study (3-4 and 5-6 weeks), and geographic location (Asia and America/Europe) (Table 3.3). As two trials involved hyperlipidemia, and two trials were conducted in Asia; these subgroups analysis have limited margin of interpretation With the exclusion of these studies, analysis of two subgroup did not appreciably alter the overall pooled estimate from the original analysis. Also, subgroups in healthy individuals demonstrated statistically significant heterogeneity for TC (I^2 = 43%) and LDL-C levels (I^2 = 63%). Likewise, subgroup analysis of studies conducted in American/ Europe demonstrated significant heterogeneity for both TC (I^2 = 55%) and LDL-C levels (I^2 = 64%). Subgroup analysis based on study duration showed that consumption of MCFAS for 5-6 weeks demonstrated significant increase in TC and HDL-C levels, however LDL-C levels did not increase. Since results obtained for TC and LDL-C levels from subgroup analysis

demonstrated significant heterogeneity, results of the subgroup analysis should be interpreted with caution.

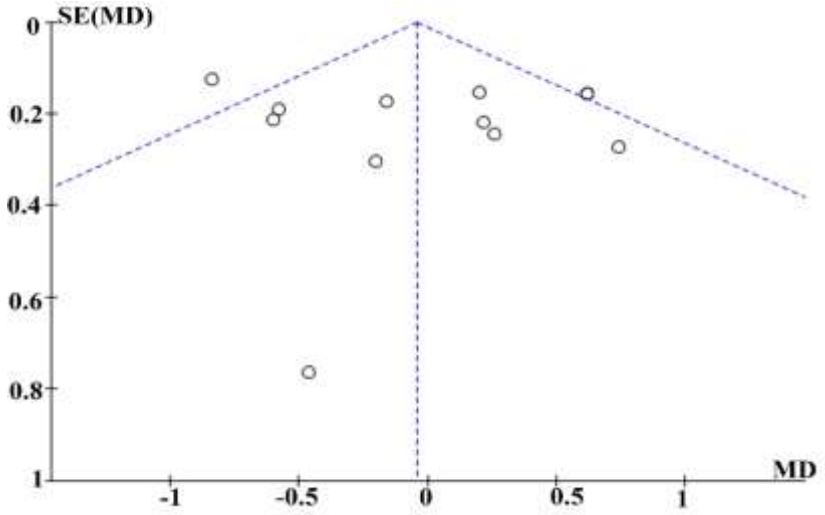
In the sensitivity analysis, removal of one study at a time did not substantially alter the results obtained for TG, LDL-C, HDL-C and TC levels compared to the original analysis. Due to the small number of included studies and lack of heterogeneity in the VLDL-C, apo A-I and apo B levels, as shown by the I^2 test, sensitivity analysis were not performed for these outcomes.

Figure 3.5

A


SE(MD)
0
0.1
0.2
0.3
0.4
0.5
MD
-0.5
-0.25
0
0.25
0.5

B


SE(MD)
0
0.2
0.4
0.6
0.8
1
MD
-1
-0.5
0
0.5
1

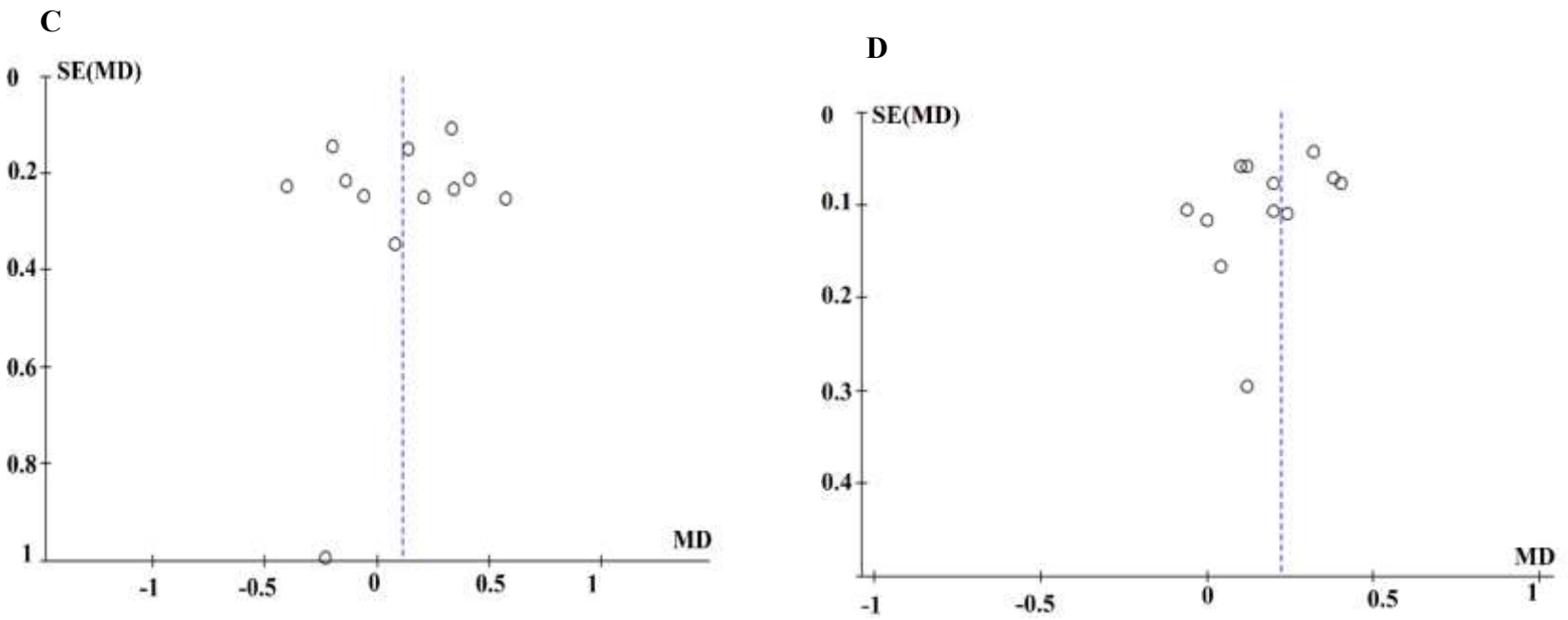


Figure 3.5. Funnel plots of (A) TG; (B) LDL-C; (C) HDL-C; (D) TC (MCFAS compared with LCSFAS) by summarizing different results of included trial in the study. Open circles represent observed published studies. The vertical line represents the pooled mean effect size. SE, Standard error; MD, mean difference

TG, Triglyceride; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; TC, Total cholesterol

Figure 3.6.

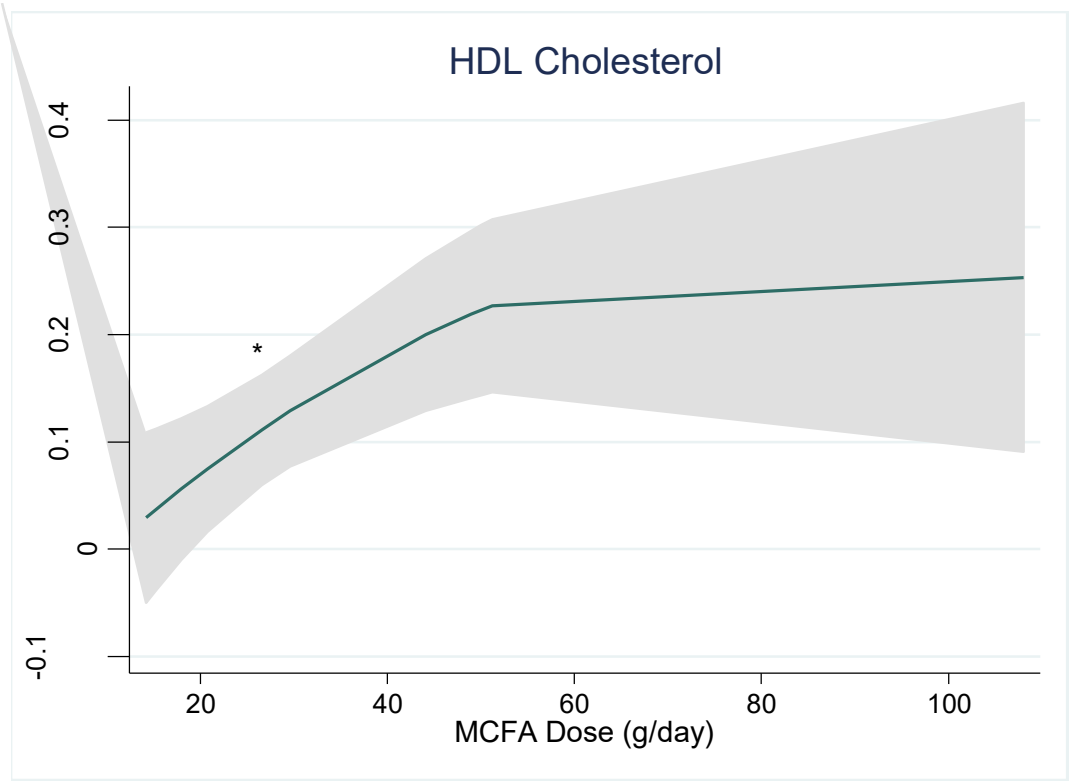


Figure 3.6. Fractional polynomial regression analysis of dose-response effect of MCFAS intake on change in HDL-C concentration. In these dose-response models, the coefficient for the MCFAS dose was an estimate of the dose-response relation between MCFAS dose and change in HDL-C concentration. The black line represents the dose-response relationship, and the grey shaded area represents the 95% CI. The regression analysis was carried out using Stata IC 14 (StataCorp LP). $*P < 0.05$

Table 3.3. Subgroup analysis comparing the effects of MCFAS and LCSFAS on lipid profile[1]

	TG		LDL-C	HDL-C	TC
	N	**Mean (95% CI) difference (mmol/L), I^2**	**Mean (95% CI) difference (mmol/L), I^2**	**Mean (95% CI) difference (mmol/L), I^2**	**Mean (95% CI) difference (mmol/L), I^2**
Washout period					
Yes	7	-0.04 (-0.11, 0.03), 3%	0.13 (-0.04, 0.30), 0%	0.13 (0.07, 0.19)[2], 0%	0.29 (0.13, 0.44)[3], 0%
No	4	-0.01 (-0.13, 0.11), 0%	-0.02 (-0.31, 0.27), 65%	0.07 (0.0, 0.14), 0%	0.02 (-0.30, 0.33), 62%
MCFAS amount					
Low (14-18g)	4	-0.08 (-0.23, 0.08), 0%	-0.07 (-0.37, 0.23), 63%	0.06 (-0.02,0.14), 0%	-0.04 (-0.38, 0.30), 7%
Moderate (20-32g)	4	0.05 (-0.06, 0.16), 0%	0.00 (-0.36, 0.37), 75%	0.11 (0.03, 0.19)[4], 0%	0.13 (-0.17, 0.44) , 62%
High (50-108g)	4	-0.04 (-0.17, 0.08), 19%	0.18 (-0.05, 0.41), 0%	0.15 (0.08, 0.22)[5], 0%	0.31 (0.13, 0.50)[6], 0%
MCFAS source					
Naturally occuring oils	10	-0.03 (-0.10, 0.03), 0%	0.01 (-0.18, 0.20), 66%	0.11 (0.07, 0.16)[7], 0%	0.12 (-0.07, 0.30), 58%
Re-esterified (manufactured) oils	2	0.18 (-0.22, 0.57), 0%	-0.12 (-0.67, 0.44), 0%	0.01 (-0.20, 0.22), 0%	0.05 (-0.59, 0.69), 0%
Participant characteristics					
Healthy	10	-0.03 (-0.10, 0.03), 0%	0.04 (-0.16, 0.24), 0%	0.11 (0.07, 0.16)[8], 0%	0.16 (-0.01, 0.33), 43%
Hyperlipidemia	2	0.10 (-0.48, 0.69), 48%	-0.23 (-0.58, 0.11), 0%	0.05 (-0.10, 0.21), 0%	-0.23 (-0.68, 0.22), 25%

Geographic location					
America and Europe	10	-0.04 (-0.12, 0.03), 0%	-0.02 (-0.24, 0.19), 64%	0.12 (0.07, 0.17)[9], 0%	0.07 (-0.13, 0.28), 55%
Asia	2	0.02 (-0.11, 0.15), 0%	02.0 (-0.01, 0.42), 63%	0.07 (-0.02, 0.17), 0%	0.23 (-0.02, 0.49), 43%
Duration of the study					
3-4 weeks	9	0.01 (-0.09, 0.11), 0%	-0.07 (-0.29, 0.14), 58%	0.10 (0.04, 0.16)[10], 0%	0.05 (-0.18, 0.28), 51%
5-6 weeks	3	-0.05 (-0.15, 0.06), 32%	0.19 (-0.01, 0.39), 0%	0.13 (0.06, 0.19)[11], 0%	0.26 (0.09, 0.42)[12], 0%
Risk of bias in studies					
High risk of bias	5	-0.02 (-0.13, 0.09), 0%	0.01 (-0.24, 0.25) 54%	0.07 (0.01, 0.14)[13], 0%	0.05 (-0.21, 0.31), 52%
Low risk of bias	7	-0.01 (-0.11, 0.08), 13%	0.00 (-0.27, 0.27), 65%	0.14 (0.08, 0.20)[14], 0%	0.16 (-0.08, 0.41), 52%

[1]The main measure of effect for a cross-over trial is the mean difference (mmol/L) and 95% CI between groups (MCFAS compared with LCSFAS) and for a parallel trial is the mean difference of the change from baseline (post intervention minus baseline) between groups (MCFAS compared with LCSFAS). The meta-analysis was performed using generic inverse variance weighting methods with a Dersimonian and Laird random effects model. Between-study heterogeneity was detected and quantified with the use of the I^2 statistic. I^2 values of approximately 25%, 50%, and 75% are considered to demonstrate low, moderate, and high levels of heterogeneity, respectively. The meta-analysis focused on outcomes with >3 trials that contributed data to pooled results.

[2,3]Significant effect of washout period ($p<0.001$); [4,5,6,7,8,9,10,11,12,13,14]significantly different from LCSFAS group ($p<0.05$); n, Number of studies; TG, Triglyceride; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; TC, Total cholesterol; MCFAS, Medium-chain saturated fatty acids; LCSFAS, Long-chain saturated fatty acid; CI, Confidence interval.

3.3.4. Assessment of risk of bias:

Studies meeting the inclusion criteria were considered in the analysis. However, many of these studies did not provide sufficient information for a clear assessment. The quality of the studies was not examined due to the lack of a validated quality scoring system for cross-over design studies. The results of the risk of bias assessment are provided in **Table 3.4**. From the 12 studies included, five were classified as having a high risk of bias (169-173), and seven as having a low risk of bias (174-179) at the primary outcome (blood lipids) level. Major possible sources of bias were carry-over and selection bias. Two studies detailed their randomization methods. Eight studies mentioned randomization but did not adequately describe the method of randomization. The remaining two studies did not use any randomization technique; hence, were classified as having a high risk of bias. From the 11 cross-over design studies, seven studies had at least a one-week washout period between interventions, and this was considered sufficient to avoid carry-over effects. The remaining four studies did not include a washout period, therefore were classified as having a high risk of bias. A low risk of reporting bias was seen in all studies in which all expected outcomes were reported. A low risk of bias was seen for missing data as all studies performed a completers analysis. Lifestyle factors that may have influenced lipid levels were considered in most studies: 11 studies checked dietary compliance; two studies monitored physical activity levels; seven studies advised participants to maintain current physical activity levels. However, data was not available for dietary compliance in one study and three studies did not report physical activity.

3.3.5. Publication bias:

Funnel plots were constructed to examine the publication bias in this meta-analysis. There was no evident asymmetry in the funnel plots, as assessed by visual inspection of funnel plots (**Figure 3.4**) and the Egger correlation test ($p \geq 0.27$), indicating a low probability of publication bias ($p = 0.41$, $p = 0.27$, $p = 0.71$, and $p = 0.65$ for TG, LDL-C, HDL -C and TC; respectively)

Table 3.1. Characteristics of included studies[1]

Author, publication year, location and reference	Participant Characteristics[2]	Study Design	Intervention compared with control	Study Duration	Outcome[3]
Voon, 2011, Malaysia (173)	n = 45:45 Men = 20% Age = 30.1 ± 8.3[4] y BMI = 23.1 ± 3.7 kg/m^2 Healthy	Randomized cross-over trial (no washout period)	20%E (~48.9 g MCFAS/d) supplied as coconut oil (20.8 g MCFAS/d) compared with an equivalent amount of palm oil	5 weeks	TG: NC LDL-C: NC HDL-C: NC TC: NC TC:HDL-C ratio: NC apo A-100: NC apo B-I: NC
Denke, 1992, United States (175)	n = 14:14 Men = 100% Age = 63 ± 5 y BMI = 25.5 ± 2.5 kg/m^2 Healthy	Randomized cross-over trail (≥ 1 week washout period)	40%E supplied as synthetic lauric oil[5] (44.1 g MCFAS/day) compared with 40%E supplied as palm oil	3 weeks	TG: NC LDL-C: +ve HDL-C: NC TC: +ve VLDL-C: NC
Reiser, 1985, United States (177)	n = 17:17 Men = 100% Age = 25.6 ± 3.5 y Healthy	Randomized cross-over trial (5 weeks washout period)	21%E (80 g/d) supplied as coconut oil (51.2 g MCFAS/d) compared with an equivalent amount of beef fat	5 weeks	TG: +ve LDL-C: NC HDL-C: +ve TC: -ve
Cater, 1997, United States (174)	n = 9:9 Men = 100% Age = 55-75 y (mean=66 y) BMI = 27 ± 5 kg/m^2 Hypercholesterolaemic	Randomized cross-over trial (≥ 1 week washout period).	43%E supplied as MCT oil[6] (108 g/day) compared with an equivalent amount of palm oil	3 weeks	TG: -ve LDL-C: NC HDL-C: NC TC: NC VLDL-C: -ve
Cox, 1995, New Zealand (169)	n = 28:28 Men = 46.4% Age, men = 55 ± 8 y Age, women = 52 ± 10 y	Randomized cross-over trial (no washout period)	~20%E (39g) supplied as coconut oil (18 g MCFAS/d) compared with an equivalent amount of butter	4 weeks	TG: +ve LDL-C: +ve HDL-C: NC

	BMI, men = 26 ± 3 kg/m^2 BMI, women = 24 ± 2 kg/m^2 Hypercholesterolaemic				TC: +ve VLDL-C : NC
Cox, 1998, New Zealand (170)	n = 37:37 Men = 56.8% Age, men = 35 ± 9 y Age, women = 39 ± 9 y BMI, men = 29.7 ± 3.5 kg/m^2 BMI, women = 26.8 ± 5.4 kg/m^2 Healthy	Sequential cross-over study (Butter diet followed by coconut oil diet; no washout period)	~20%E (39g) supplied as coconut oil (18 g MCFAS/d) compared with an equivalent amount of butter	4 weeks	TG: NC LDL-C: +ve HDL- C: NC TC: NC VLDL-C: NC apo A-I: NC apo B: +ve
Temme, 1996, Netherland (172)	n = 30:30 Men = 40.0% Age = 20-60 y (mean=41 y) BMI = 19-30 kg/m^2 (mean = 25 kg/m^2) Healthy	Cross-over (>2 week washout period)	28%E (70.4g) supplied as high lauric acid oil blend (75% palm kernel oil + 25% high oleic sunflower oil) (26.6 g MCFAS/d) compared with an equivalent amount of palmitic acid oil blend (55% diary fat + 36% palm stearin + 9% sunflower oil)	6 weeks	TG: NC LDL-C: NC HDL-C: +ve TC : -ve HDL: LDL ratio: NC apo A-I: -ve apo B: NC
Schwab, 1995, Finland (178)	n = 15:15 Men = 0% Age = 23 ± 1.2 y BMI = 21.4 ± 0.5 kg/m^2 Healthy	Randomized cross-over trial (2 week washout period)	4%E supplied as lauric acid blend (16-26g coconut oil + 5-8g rapeseed oil + 3-4.5g olive oil + 2-3.5g sunflower oil) (mean intake: 14.2g MCFAS/d) compared with an equivalent amount of palmitic acid oil blend (22-33g palm oil + 2-5g soybean oil)	4 weeks	TG: NC LDL-C: NC HDL-C: NC TC: NC VLDL-C: NC apo A-I: NC apo B: NC
Sundaram , 1994, Malaysia (171)	n = 17:17 Men = 100% Age = 21 ± 1.1 y BMI = 20.1 ± 1.8 kg/m^2 Healthy	Double-blind randomized cross-over trial (no washout period)	5%E supplied as lauric/myristic oil blend (30% coconut oil + 35% palm kernel oil + 35% canola oil) (15.3g MCFAS/d) compared with an equivalent amount of palmitic oil blend	4 weeks	TG: NC TC: -ve LDL: -ve HDL-C: +ve LDL-C/HDL-C ratio: NC VLDL-C: NC

			(55% palm stearin + 25% palm oil + 10% palm kernel oil + 10% corn oil)		apo A-1: NC apo B: NC
Heber, 1992, United States (176)	n = 9:9 Men = 100% Age = 22-43 y Healthy	Randomized cross-over trial (2 week washout period)	17.5%E (46.9 g) supplied as coconut oil (29.6g MCFAS/d) compared with an equivalent amount of palm oil	3 weeks	TC: -ve LDL-C: NC HDL-C: NC TC: NC apo A1: NC apo B: NC
Tholstrup, 1995, Sweden (179)	n = 15:15 Men = 100% Age = 22-30 (mean: 24.9 y) BMI = 20.4-26.4 kg/m^2 (mean: 23.2 kg/m^2) Healthy	Randomized cross-over trial (1-2 months washout period)	36%E (140.1g) supplied as a high myristic/lauric acid blend (palm kernel oil + high oleic sunflower oil) (49.0g MCFAS/d) compared with an equivalent amount of palm oil	3 weeks	TC: -ve LDL-C: -ve HDL: +ve TG: NC VLDL: NC LDL-HDL-C ratio: +ve Lp(a): NC
Khaw K-T, 2018, United Kingdom (180)	n = 29:33 Men = 35.6% Age = 50–75 y (mean: 60.5 y) BMI = 25.15 kg/m^2 Healthy	Randomized parallel trial	19%E (50 g) supplied as a coconut oil (31.5 g MCFAS/d) compared with an equivalent amount of butter	4 weeks	TC: -ve LDL-C: -ve HDL-C: +ve TG: NC

[1]TC, Total cholesterol, LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; *TG,* Triglyceride; TC:HDL, total cholesterol-to-HDL ratio; VLDL, Very low-density lipoprotein cholesterol; HDL: LDL-C, HDL-to-LDL ratio; LDL: HDL, LDL-to-HDL ratio; apo A-I, Apolipoprotein A-I; apo B, Apolipoprotein B; apo A-100, Apolipoprotein A-100; Lp(a), Lipoprotein (a); MCFAS, Medium-chain saturated fatty acids; NC, no change; +ve, favors intervention; -ve, favors control.
[2]n = intervention: control
[3]Outcomes denotes effect of intervention on lipid and lipoprotein levels
[4]Means ± SDs
[5]Trilaurin; Proctor and Gamble company, Cincinnati
[6]MCT oil; Mead Johnson, Evansville, IN

Table 3.2. Summary of outcome measures showing differential effects of MCFAS and LCSFAS on blood lipids[1]

Reference	MCFAS amount/d	Intervention (mmol/L)	Control (mmol/L)	Mean differences (MCFAS minus LCSFAS) in blood lipid (mmol/L) (95% CI)	Limitation
Voon (173)	20.8 g/d	TG: 0.90 ± 0.39 LDL-C: 3.30 ± 0.75 HDL-C: 1.37 ± 0.30 TC: 4.95 ± 0.69	TG: 0.85 ± 0.31 LDL-C: 3.20 ± 0.71 HDL-C: 1.31 ± 0.26 TC: 4.81 ± 0.74	TG: 0.05 (-0.10, 0.20) LDL-C: 0.10 (-0.20, 0.40) HDL-C: 0.06 (-0.06, 0.18) TC: 0.14 (-0.16, 0.44)	-
Denke (175)	44.1 g/d	TG: 1.06 ± 0.71 LDL-C: 3.70 ± 2.13 HDL-C: 0.96 ± 0.78 TC: 4.94 ± 2.80	TG: 1.06 ± 0.71 LDL-C: 3.93 ± 1.91 HDL-C: 0.90 ± 0.78 TC: 5.17 ± 2.43	TG: 0.00 (-0.53, 0.53) LDL-C: -0.23 (-1.73, 1.27) HDL-C: 0.06 (-0.52, 0.64) TC: -0.23 (-2.18, 1.72)	No energy intake reported; MCFAS amount estimated from average adult energy intake.
Reiser (177)	51.2 g/d	TG: 0.88 ± 0.17 LDL-C: 2.84 ± 0.43 HDL-C: 1.19 ± 0.12 TC: 4.34 ± 0.32	TG: 0.99 ± 0.16 LDL-C: 2.53 ± 0.48 HDL-C: 1.03 ± 0.13 TC: 4.01 ± 0.32	TG: -0.11 (-0.22, -0.00) LDL-C: 0.31 (0.00, 0.62) HDL-C: 0.16 (0.07, 0.24) TC: 0.34 (0.12, 0.55)	-
Cater (174)	108 g/d	TG: 1.75 ± 0.80 LDL-C: 4.27 ± 0.59 HDL-C: 0.91 ± 0.31 TC: 5.87 ± 0.75	TG: 1.35 ± 0.42 LDL-C: 4.37 ± 0.70 HDL-C: 0.91 ± 0.16 TC: 5.79 ± 0.72	TG: 0.40 (-0.19, 0.99) LDL-C: -0.10 (-0.70, 0.50) HDL-C: 0.00 (-0.23, 0.23) TC: 0.08 (-0.60, 0.76)	No energy intake reported; MCFAS amount estimated from average adult energy intake.
Cox (169)	18 g/d	TG: 1.8 ± 1.0 LDL-C: 4.2 ± 0.8 HDL-C: 1.5 ± 0.4 TC: 6.4 ± 0.8	TG: 2.0 ± 1.3 LDL-C: 4.5 ± 0.8 HDL-C: 1.4 ± 0.4 TC: 6.8 ± 0.9	TG: -0.20 (-0.81, 0.41) LDL-C: -0.30 (-0.72, 0.12) HDL-C: 0.10 (-0.11, 0.31) TC: -0.40 (-0.85, 0.05)	-

Cox (170)	18 g/d	TG: 1.61 ± 0.93 LDL-C: 3.79 ± 0.75 HDL-C: 1.21 ± 0.27 TC: 5.47 ± 0.91	TG: 1.86 ± 0.89 LDL-C: 4.08 ± 0.89 HDL-C: 1.16 ± 0.24 TC: 5.61 ± 0.96	TG: -0.25 (-0.66, 0.16) LDL-C: -0.29 (-0.67, 0.09) HDL-C: 0.05 (-0.07, 0.17) TC: -0.14 (-0.57, 0.29)	-
Temme (172)	26.6 g/d	TG: 1.05 ± 0.50 LDL-C: 3.84 ± 0.99 HDL-C: 1.59 ± 0.45 TC: 5.90 ± 1.02	TG: 1.11 ± 0.54 LDL-C: 3.71 ± 0.91 HDL-C: 1.47 ± 0.40 TC: 5.69 ± 0.93	TG: -0.06 (-0.32, 0.20) LDL-C: 0.13 (-0.35, 0.61) HDL-C: 0.12 (-0.10, 0.34) TC: 0.21 (-0.28, 0.70)	-
Schwab (178)	14.2 g/d	TG: 0.87 ± 0.35 LDL-C: 2.85 ± 0.46 HDL-C: 1.49 ± 0.27 TC: 4.85 ± 0.62	TG: 0.87 ± 0.31 LDL-C: 2.93 ± 0.50 HDL-C: 1.52 ± 0.31 TC: 4.91 ± 0.74	TG: 0.00 (-0.24, 0.24) LDL-C: -0.08 (-0.43, 0.27) HDL-C: -0.03 (-0.24, 0.18) TC: -0.06 (-0.55, 0.43)	-
Sundaram (171)	15.3 g/d	TG: 1.00 ± 0.42 LDL-C: 2.70 ± 0.46 HDL-C: 1.18 ± 0.25 TC: 4.37 ± 0.62	TG: 1.08 ± 0.37 LDL-C: 2.39 ± 0.46 HDL-C: 1.08 ± 0.20 TC: 3.96 ± 0.62	TG: -0.08 (-0.35, 0.19) LDL-C: 0.31 (0.00, 0.62) HDL-C: 0.10 (-0.05, 0.25) TC: 0.41 (-0.01, 0.83)	-
Heber (176)	29.6 g/d	TG: 1.24 ± 0.78 LDL-C: 3.34 ±0.62 HDL-C: 1.08 ±0.31 TC: 5.04 ± 0.54	TG: 0.89 ± 0.20 LDL-C: 2.97 ± 0.54 HDL-C: 1.06 ± 0.39 TC: 4.47 ± 0.54	TG: 0.35 (-0.18, 0.88) LDL-C: 0.11 (-0.32, 0.54) HDL-C: 0.03 (-0.30, 0.35) TC: 0.57 (0.07, 1.07)	-
Tholstrup (179)	49 g/d	TG: 0.74 ± 0.31 LDL-C: 3.07 ± 0.66 HDL-C: 1.19 ± 0.23 TC: 4.42 ± 0.69	TG: 0.73 ± 0.19 LDL-C: 2.96 ± 0.54 HDL-C: 0.99 ± 0.19 TC: 4.08 ± 0.58	TG: 0.01 (-0.17, 0.19) LDL-C: 0.11 (-0.32, 0.54) HDL-C: 0.20 (0.05, 0.35) TC: 0.34 (-0.12, 0.80)	-

Khaw K-T[2] (180)	31.5 g/d	TG: 0.07 ± 0.58 LDL-C: -0.09 ± 0.49 HDL-C: 0.28 ± 0.29 TC: 0.22 ± 0.55	TG: -0.001 ± 0.36 LDL-C: 0.33 ± 0.48 HDL-C: 0.09 ± 0.27 TC: 0.42 ± 0.59	TG: 0.07 (-0.18, 0.32) LDL-C: -0.42 (-0.66, -0.18) HDL-C: 0.19 (0.05, 0.33) TC: 0.20 (-0.49, 0.09)

[1]The summary measure for cross-over trial is the mean difference (mmol/L) and 95% CI between groups (MCFAS compared with LCSFAS). [2]The principal summary measure for a parallel trial is the mean difference of the change from baseline (post intervention minus baseline) between groups (MCFAS compared with LCSFAS). The meta-analysis was performed using generic inverse variance weighting methods with a Dersimonian and Laird random effects model. Values are reported as means ± SDs for all plasma lipid concentrations

TG, Triglyceride; LDL-C, Low-density lipoprotein cholesterol*;* HDL-C*,* High-density lipoprotein cholesterol; TC, Total cholesterol; MCFAS, Medium-chain saturated fatty acids; LCSFAS, long-chain saturated fatty acids; CI, confidence interval

Table 3.4. Risk of bias of included studies

Source	Sequence generation	Carry-over effects	Allocation concealment	Blinding	Missing data	Selective report	Overall effect
Cater, 1997 (174)	Unclear	Low	Unclear	High	Low	Low	Low
Cox, 1998 (170)	High	High	Unclear	High	Low	Low	High
Cox, 1995 (169)	Low	High	Unclear	High	Low	Low	High
Denke, 1992 (175)	Unclear	Low	Unclear	High	Low	Low	Low
Heber, 1992 (176)	Unclear	Low	Unclear	High	Low	Low	Low
Reiser, 1985 (177)	Unclear	Low	Unclear	High	Low	Low	Low
Schwab, 1995 (178)	Unclear	Low	Unclear	High	Low	Low	Low
Sundaram, 1994 (171)	Unclear	High	Unclear	Low	Low	Low	High
Temme, 1996 (172)	High	Low	Unclear	High	Low	Low	High
Tholstrup, 1995 (179)	Unclear	Low	Unclear	High	Low	Low	Low
Voon, 2011 (173)	Low	High	Unclear	Low	Low	Low	High
Khaw K-T, 2018 (180)	Low	n/a	Unclear	Low	Low	Low	Low

Methodological quality of the studies included in this systematic review. n/a, not applicable

3.4. DISCUSSION

This systematic review and meta-analysis aimed to determine whether MCFAS and LCSFAS have differential effects on blood lipid and lipoprotein levels. To our knowledge, this is the first meta-analysis to quantify the effect of a MCFAS diet compared to a LCSFAS diet on comprehensive lipid parameters. This study demonstrates that MCFAS increase plasma HDL-C and apo A-I levels compared to LCSFAS.

The findings of this review support the results from the Nurses' Health Study, in which intake of MCFAS was not associated with coronary heart disease, whereas intake of LCSFAS was associated with increased risk (156). High levels of HDL-C are inversely correlated with the risk of CVD. Epidemiological evidence shows that when HDL-C levels are increased by 0.025 mmol/L, the associated risk of developing CVD is reduced by 2-3% (152). In this meta-analysis, MCFAS diets compared to LCSFAS diets increased plasma HDL-C concentrations by 0.11 mmol/L, which would suggest that reduction in CVD risk following MCFAS consumption may be clinically relevant. apo A-I is the major protein component of HDL particles (181); thus, plasma apo A-I concentrations strongly correlate with plasma HDL-C concentrations (182). In this systematic review, both apo A-I and HDL-C levels were significantly higher after MCFAS compared to LCSFAS, providing support for the validity of this outcome.

MCFAS selectively upregulate intestinal synthesis of apo A-I level (183), which interacts with ATP binding cassette transporter A1 (ABCA1) (184). The interaction of apo A-I and ABCA1 mediate the efflux of cellular cholesterol to the lipid-free apo A-I (184, 185), thereby promoting reverse cholesterol transport (RCT) and biogenesis of HDL particles (185). Furthermore, MCFAS enhances the hepatic messenger RNA (mRNA) expression of ABCA1 which plays an important role in the biogenesis of HDL particles (186).

Amounts of MCFAS varied considerably from 4% percent energy (14.2 g/day) up to 36% energy (140.1 g/day). Subgroup analysis from this review showed that moderate-high amounts (20-140 g/d) MCFAS were required to increase HDL-C levels. Lower amounts of MCFAS were less effective in increasing HDL-C level, however this could be due to the limited number of studies in the analysis.

In spite of some inconsistent results from individual clinical trials, pooling data from 12 studies in this analysis showed that MCFAS significantly increase HDL-C levels compared to to LCSFAS. Heterogeneity within the meta-analysis may have arisen from the methodology of individual studies. Trials that failed to ensure a washout period may have resulted in a false

result due to a carry-over effect (187). The use of re-esterified (MCT or trilaurin oil) versus naturally occuring MCFAS (coconut or palm kernel oil) in the interventions may have influenced the study outcome. It would appear that naturally occuring but not re-esterified MCFAS increase HDL-C levels. However, result on re-esterified fats should be interpreted with caution because the analysis was based on only two studies. Manufactured fats have been shown to elicit a different metabolic response than that induced by natural fats containing the same fatty acids (188, 189), it is possible that this is the case for MCFAS and requires further investigation. The majority of studies (10 out of 12) included in the meta-analysis were conducted in healthy people, thus limiting the generalizability of results to other populations. As dyslipidemia is a key risk factor for CVD, further research is required to determine whether MCFAS compared to LCSFAS have differential effects in people with hypercholesterolemia and/or dyslipidemia. Of 12 papers included in the meta-analysis, only two research papers were recent (less than 10 years old) and ten papers were older (more than 20 years old). Measuring blood lipids by Cholestech LDX analyser showed better reproducibility than the CardioChek PA analyser when compared aginst gold standard method (1). Lifestyle changes is often the most choice for improving blood lipids; however, lipid lowering drugs may prevent or treat abnormalities in plasma lipid levels. The most compelling scientific evidence that currently available lipid lowering therapy is beneficial for atherosclerosis and risk of coronary artery disease comes from interventional trials using the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, are the most potent lipid-lowering agents currently available (2). Before lovastatin were available in 1987, medicines such as bile acid sequestrants such as cholestyramine and colestipol, nicotinic acid, the fibrates, and probucol were used to lowers blood lipid levels. However, these lipid lowering agents have limited efficacy or tolerability [3, 4]

Two studies were conducted in Malaysia involving coconut oil supplementation in addition to their usually high intake as part of the regular diet, however, 10 studies were conducted in countries where use of coconut oil is not part of the regular diet. This needs to be considered when interpreting the results. A key limitation worthy of note was that seven studies presented a high risk of bias, mainly due to carry-over bias and sequence generation bias. Furthermore, when there is industry funding, guiding principles such as the control of the research design by the study investigator, freedom to publish research findings and disclosure of the financial interest is important. In this systematic review, three industry funded trials were listed as co-authors, therefore it is assumed that they contributed in the research design, interpretation of the data and approval for the final manuscript, which can conflict with the guiding principles

mentioned above. In eight studies, data on alcohol consumption, which might influence HDL-C levels, were not considered. Cross-over trials only reported end values so changes from baseline could not be described, thus the effect of MCFAS on overall CVD risk is difficult to establish in these studies.

Previous studies indicated that LDL-C and TC are important risk factor for CVD (190). In this meta-analysis, although not significant, the trend towards higher TC by MCFAS compared to LCSFAS is likely driven by the higher HDL-C, as no significant change was observed in the LDL-C levels. However, as results obtained for TC and LDL-C levels from original and subgroup analysis demonstrated significant heterogeneity, no meaningful conclusions from this analysis can be drawn, therefore results of the subgroup analysis should be interpreted with caution.

Although the limitations of the individual studies discussed above cannot be ignored, this meta-analysis provides a consolidated evidence for the differential effects of MCFAS on HDL-C levels. The review employed a comprehensive approach to identify relevant English-language studies, and the meta-analysis included data from all relevant studies irrespective of the experimental design. In addition, five out of seven outcomes demonstrated very little or no heterogeneity. Furthermore, the present systematic review contained mainly cross-over studies, which are generally known to have a more-robust design because of reduced intra-participant variability (191). Finally, the lack of significant asymmetry in the funnel plots of all variables as assessed by visual inspection of funnel plots and statistical assessment (168) demonstrated that our findings are unlikely to be affected by publication bias.

Further investigation of the metabolic effects of varying chain length of fatty acids will provide clarity around the current controversy in the literature surrounding the cardiovascular health effects of SFA. Further well-designed randomized-controlled studies with a longer durations (over 6 weeks and with washout periods of at least one week) are needed to validate the differential effects of MCFAS and LCSFAS on blood lipid profiles and to elucidate the possible mechanisms involved. Additional studies to estimate the optimal amount of MCFAS consumption are required to help appraise the overall longer-term benefits of MCFAS consumption on health and CVD outcome. However, the increase in MCFAS as a replacement for MUFA or PUFA in imrpoving blood lipids is yet to be investigated.

In conclusion, consumption of MCFAS as part of energy maintenance diet may result in higher HDL-C levels as compared to LCSFAS highlighting the importance of considering chain length when measuring the effect of dietary SFA on lipid profile. Nutritional guidelines on

SFA and CVD may require reappraisal if the current evidence favouring MCFAS can be further validated.

Chapter 4:

Medium chain fatty acids lower postprandial lipemia: a randomized crossover trial

The content described by this chapter has been submitted as the following manuscript:

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Statement of authorship in appendix 2

Research in human studies have shown that SFA of different chain lengths displays different metabolic properties and functions. Therefore, our aim in Chapter 4 was to to examine the differential effect of chain length of SFA (SCFA, MCFAS and LCSFAS) on postprandial blood lipid levels.

Abstract

Background: Dyslipidaemia is an established major modifiable risk factor for cardiovascular disease. Epidemiological and intervention studies have linked saturated fatty acids with elevated levels of low-density lipoprotein cholesterol (LDL-C) and increased risk of CVD. However, the effects of variance in chain length of saturated fatty acids (SFA) on postprandial lipemia in humans are not well elucidated.

Objective: The aim of this study was to investigate the impact of short, medium and long chain SFA on postprandial blood lipids in healthy men and women.

Methods: Sixteen healthy volunteers consumed test biscuits containing 40 grams of either butter (BB), coconut oil (CB) or lard (LB) in a single-blinded, randomized crossover design. Blood samples were collected fasting and 2, 3, 4, and 6 hours postprandially and assessed for blood lipids (total cholesterol, TC; high-density lipoprotein cholesterol, HDL-C; LDL-C and triglyceride, TG).

Results: The postprandial TG response following CB was 59.8% lower than after BB ($p<0.01$) and 58.8% lower than LB ($p<0.01$), although no difference was observed between the BB and the LB responses. The net area under the LDL-C concentration curve was significantly larger after consumption of the CB compared to the BB, despite no significant differences in postprandial net area under the TC and HDL-C concentration curves.

Conclusion: Consumption of medium-chain SFA as CB resulted in lower postprandial TG excursions compared to consumption of short-chain SFA present in BB and long-chain SFA present in LB, despite identical fat and caloric content. These results suggest that saturated fats differ in their potential to elevate postprandial lipid levels, and that coconut oil, a rich source of medium chain fatty acids may not be as hyperlipidemic as animal fats rich in long chain SFA.

ANZCTR identifier: 12617000903381
Keywords: saturated fatty acids, postprandial, triglycerides, butter, coconut oil, lard

4.1. Introduction

Dietary saturated fatty acids (SFA) have frequently been linked to detrimental health effects in the literature over the last eight decades (151). However, heterogeneous results have been reported in both epidemiological and interventional studies suggesting that this relationship may be complex*;* some studies have demonstrated that SFA are positively associated with dyslipidaemia and chronic disease risk (192, 193), whereas other studies have found no association (194-198) or even a negative association (199, 200). This study aimed to identify whether the heterogeneity could be attributable to SFA chain-length, by investigating the effect of different SFAs on post-prandial blood lipids.

SFA are classified into short, medium and long-chain depending on their number of carbon atoms. Short-chain SFA (SCFA, 2-4 carbons long) are found in lower proportions in the diet than either medium-chain SFA (MCFAS, 6-12 carbons long) or long-chain SFA (LCSFAS, >12 carbons). SCFA are present in small proportions in few dietary fats (e.g. butter with 3% butyric acid) and are also produced by the gut microbiome in the large bowl (201). MCFAS are present in large amounts in coconut oil, approximately 63% (27, 202). LCSFAS are present in dairy products (e.g. butter) as well as in animal fats (e.g. tallow and lard) (27, 203). A small number of studies have compared the effect of SFA with different chain lengths on fasting blood lipid levels. A study using an animal model reported lower fasting triglycerides (TG) and higher high density lipoprotein cholesterol (HDL-C) concentrations after consumption of coconut oil for 3 weeks, compared to an equivalent amount of beef tallow (159). In a 5-week interventional study, consumption of coconut oil significantly increased fasting HDL-C and lowered TG concentrations compared to equivalent amounts of beef tallow in healthy human subjects (204). Notably, we have earlier shown that consumption of MCFAS results in high plasma concentration of HDL-C and apo A-I (205).

SCFA and MCFAS are absorbed directly through the villi of the intestinal mucosa and transported to the liver via portal circulation. In contrast, LCSFAS follow a more complex metabolic pathway that encompasses the syntesis of chylomicrons in the intestinal villi (5, 35, 43, 206). The elevation of plasma triglycerides in the post-meal state is mainly derived from an increased production of intestinally-derived chylomicrons particles (207, 208). The magnitude and the duration of postprandial lipemia, characterized by changes in plasma triglyceride levels following consumption of a meal, has been identified as an independent risk factor for cardiovascular disease (CVD) (76). Since the majority of each 24 hours is spent in

the postprandial state (76), it is important to characterize the ways in which different dietary fatty acids influence postprandial lipid metabolism. SFA-induced postprandial lipemia is of longer duration than that of polyunsaturated fatty acids (PUFA) of the omega-6 (n-6) family (76). Studies have compared the effects of SFA, polyunsaturated fatty acids and monounsaturated fatty acids (MUFA) on postprandial lipemia, but there are no studies of SFA with different chain lengths (134, 137).

It has been reported that MCFAS derived from coconut oil are not incorporated into chylomicrons, as are LCSFAS, and do not play a part in the biosynthesis and transport of cholesterol (5, 35, 43, 206). As a result, it could be hypothesised that consumption of MCFAS would not increase postprandial lipid levels as significantly as LCSFAS (76). To date, the postprandial effect of consuming SFA of different chain lengths on lipemia has not been directly investigated. This study was designed to evaluate the differential effects of foods containing SCFA (butter), MCFAS (coconut oil) or LCSFAS (lard) on postprandial blood lipids.

4.2. Methods

4.2.1. Study Population

The study population consisted of 16 healthy adults (8 women and 8 men) aged between 18 and 45 years. The study was approved by the University of Newcastle Human Research Ethics Committee (approval # H-2016-0429) and registered with the Australia New Zealand Trial registry as ACTRN12617000903381. Participants were recruited from the public by means of advertisements and flyers. Participants were excluded if they were using lipid-lowering drugs; had consumed fish oil supplements regularly within the past month; had regular consumption of two or more fish meals a week over the past month; had any history of congestive heart failure, stroke, myocardial infarction, coronary artery bypass graft, or established atherosclerotic disease; a history of diabetes, gastrointestinal or liver disease; were current smokers; or were pregnant or breastfeeding.

4.2.2. Study design and experimental protocol

This was a randomized, cross-over, single-blinded design study with three experimental test days separated by at least a week of washout period. All subjects were randomly assigned to

each of the interventional foods using a computer-based procedure (209). On the day preceding each test, participants were advised to maintain their usual dietary intake and to abstain from alcohol and strenuous physical activity. On the test day, participants visited the University of Newcastle after an overnight fast of at least 10 hours. Female subjects were not studied during menses. At their first visit, participants had their anthropometric (height, weight, percentage body fat, waist circumference) and blood pressure measurements taken and completed questionnaires on their eating attitudes (EAT-26) (210) and a physical activity (International Physical Activity Questionnaire, IPAQ) (211). At each visit, participants were asked to complete a 24-hour food recall; dietary information was analysed by FoodWorks 8.0.3551 (Xyris software). The test foods consisted of biscuits containing either 40g of butter fat (BB), 40g coconut oil (CB) or 40g lard (LB). The butter biscuits contained 61g carbohydrates, 7g protein and 37g fat (24g saturated, 7g monounsaturated, 1g polyunsaturated fat); the coconut biscuits contained 66g carbohydrates, 7g protein and 39g fat (35g saturated, 0.6g monounsaturated, 0.6g polyunsaturated fat) and the lard biscuits contained 66g carbohydrates, 7g protein and 39g fat (20g saturated, 14g monounsaturated, 1.5 g polyunsaturated fat). Butter cosist of butyric acid (SCFA) and long-chain SFA (myristic, palmitic and stearic acid). Coconut oil contains medium chainm SFA (caprylic acid, decanoic acid and lauric acid) and long chain SFA (myristic and palmitic acid). Lard contains long chain SFA (palmitic, stearic acid and myristic acid) (212, 213).

Subjects received each of the three types of biscuit in different study visits in random order. All test biscuits were consumed within 15min and served with 250ml of water. No other food was ingested during the study period. Participants were allowed water *ad libidum* on their first test day; the volume of water was measured and replicated on the subsequent test days. Blood samples were collected after an overnight fast and 2, 3, 4 and 6 hours after consuming the biscuits and were assessed for blood lipid profiles [total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), HDL-C and TG].

4.2.3. Measures

Participant height was measured using a stadiometer; body weight and composition were measured using the bioelectric impedance method (InBody 230, Biospace Co. Ltd. Seoul, Korea) and blood pressure was measured using an automated sphygmomanometer (*Pulsecor Cardioscope*). Capillary blood samples were collected in heparinised capillary tubes using

finger stick with the first drop of expressed blood discarded. These samples were assessed for blood lipid profiles (TC, LDL-C, HDL-C, and TG) using Cardio Check PA (PTS Diagnostics, USA).

4.2.4. Statistical analysis

All data were expressed as mean ± standard deviation unless otherwise specified. Postprandial blood lipid concentration changes were used to calculate the net area under the curve (AUC) using the trapezoidal rule for the 360-minute collection period. Within each test day, one-way analysis of variance with repeated measurements was applied to analyse differences between baseline and subsequent time points. The change from baseline to each time point was compared using paired *t* test and Wilcoxon signed-rank test for parametric and non-parametric data, respectively. The difference between net AUCs on different test days was compared using a paired Student's *t* test and Wilcoxon signed-rank test for parametric and non-parametric data, respectively. The effect of the type of fat on blood levels of glucose and lipids over the duration of the test period were compared using a two-way analysis of variance with repeated measurements (interaction biscuit × time). Differences in postprandial lipid concentrations at different time points between test fats were compared using a paired Student's *t* test and Wilcoxon signed-rank test for parametric and non-parametric data, respectively. A p-value <0.05 was considered statistically significant. All analyses were carried out using Stata IC 14 (StataCorp LP).

4.3. Results

Sixteen healthy adults (8 males and 8 females) completed the study with no missing data points (Figure 1). The characteristics of the study population are presented in Table 4.1. All participants attended the three test days and no adverse effects were reported. The mean energy intake of subjects 24 hours before the 3 test days was not statistically different (Table 4.2). Likewise, fasting concentrations of TC, LDL-C, TG, and HDL-C were not statistically different between test days (Table 3). The mean time taken to ingest the BB (12.4 ± 1.6 min), the CB (12.8 ± 2 min) and the LB (13 ± 2.09 min) were similar.

Changes in postprandial TG concentrations are shown in Figure 4.2 and Table 4.3. There were time-dependent changes in TG concentrations after the CB, BB, and LB ($p<0.001$, for all interventions). There was a significant time × biscuit interaction ($p<0.01$) for TG concentrations during the 6-hour postprandial period. TG levels peaked 3-hours after the BB and the LB with the maximum mean difference from baseline being 0.85 mmol/L and 0.88 mmol/L respectively. The peak TG concentration following the CB also occurred at 3-hours, but was significantly lower with a maximum mean difference from baseline of 0.29 mmol/L. The TG concentration following the CB was also significantly lower than the BB and the LB at 2-hours ($p<0.01$) and 4-hours ($p<0.01$). The net AUC for TG change was 1.72 mmol/L and 1.64 mmol/L at these time points, this was significantly higher than after consumption of the BB (59.8% higher; $p<0.01$) and the LB (58.8% higher; $p<0.01$). TG levels returned to baseline 6 hours after the consumption of the CB (0.13 mmol/L), whereas TG levels remained significantly higher than baseline values 6 hours following consumption of the BB (0.19 ± 0.34 mmol/l; $p<0.05$) and the LB (0.l9 ± 0.27 mmol/L; $p<0.05$).

Postprandial change in LDL-C concentrations are shown in Figure 4.2 (A, B) and Table 4.3. There were time-dependent changes in LDL-C following BB ($p<0.01$), CB ($p<0.05$) and LB ($p<0.05$) without significant biscuit x time interaction effects ($p = 0.24$). The LDL-C change net AUC was significantly larger following the CB than following the BB (0.28 vs. -0.84 mmol/L; $p<0.05$). LDL-C concentrations following the CB were significantly higher than following the LB 3 hours after consumption ($p<0.05$), and higher than the BB after 3 ($p<0.05$) and 4 hours ($p<0.05$). When comparing the three test biscuits, no significant differences were observed in HDL-C and TC change net AUC (Table 4.3).

Table 4.1. Participant characteristics at baseline measured only at the first visit[1]

Characteristic	Values
Sex, *n* (%)	
Male	8 (50)
Female	8 (50)
Ethnic group, *n* (%)	
Caucasian	5 (31)
Asian	11 (69)
IPAQ score[a] 1 (low) *n* (%)	16 (100)
Age, years	26.2 ± 8.4
Weight, kg	64.7 ± 10
Body mass index, kg/m^2	23.7 ± 2.8
Body fat, %	26.7 ± 8.1
Waist circumference, cm	82.2 ± 11.2
Systolic BP, mmHg	109 ± 8
Diastolic BP, mmHg	70 ± 6

[1]Values are reported as means ± standard deviation for continuous measures and as n (%) for categorical measure

Table 4.2. Fasting blood lipids and nutrient intake 24 hours prior to each postprandial intervention[1]

Plasma Lipid (mmol/L)	**BB**	**CB**	**LB**
Triglyceride	1.04 ± 0.35	1.02 ± 0.50	1.12± 0.49
Total cholesterol	4.07 ± 0.59	4.20 ± 0.67	4.02 ± 0.61
HDL cholesterol	1.18 ± 0.30	1.22 ± 0.33	1.18 ± 0.29
LDL cholesterol	2.41 ± 0.46	2.51 ± 0.53	2.33 ± 0.45
Total cholesterol/HDL ratio	3.58 ± 0.73	3.56 ± 0.78	3.58 ± 0.88
Nutrients intake			
Energy, MJ	9.41 ± 2.98	9.55 ± 2.92	9.69 ± 2.79
Protein, g	102.7 ± 55.4	98.2 ± 39.6	101.6 ± 43.9
Carbohydrate, g	258.2 ± 69.7	256.5 ± 88	267.1 ± 54.9
Total fat, g	78.6 ± 40.1	88.9 ± 42.8	84.4 ± 48.3
Saturated fat, g	27 ± 15.2	33.4 ± 24.1	29.5 ± 16.4
Monounsaturated fat, g	30.8 ± 20.9	33.9 ± 17.6	33.6 ± 22.1
Polyunsaturated fat, g	14.1 ± 6.4	13.8 ± 8.0	14.3 ± 10.1
Cholesterol, mg	249 ± 165	238 ± 164	206 ± 151

[1]Values are reported as means ± standard deviation. BB, Butter biscuit; CB, Coconut oil biscuit; LB: Lard biscuit; HDL, high density lipoprotein; LDL-C, low density lipoprotein cholesterol.

Table 4.3. Fasting lipid profile at baseline, changes over 6 hours postprandially and net area under the curve for change values (**Δ** netAUC) following consumption of BB, CB or LB[1].

Blood lipids (mmol/L)	**Baseline**	**Δ2h**	**Δ3h**	**Δ4h**	**Δ6h**	**Δ net AUC**
TG						
BB	1.04 ± 0.35	0.58 ± 0.37^{b*}	0.85 ± 0.50^{b*}	0.65 ± 0.36^{b*}	0.19 ± 0.34*	2.88 ± 1.71^{a}
LB	1.12 ± 0.49	0.61 ± 0.37^{b*}	0.88 ± 0.51^{b*}	0.55 ± 0.37^{b*}	0.19 ± 0.27*	2.8 ± 1.59^{a}
CB	1.02 ± 0.50	0.24 ± 0.18^{a*}	0.29 ± 0.21^{a*}	0.25 ± 0.34^{a*}	0.13 ± 0.19	1.16 ± 1.04^{b}
TC						
BB	4.07 ± 0.59	0.04 ± 0.23	0.14 ± 0.18*	0.14 ± 0.32	0.16 ± 0.35	0.55 ± 0.91
LB	4.02 ± 0.61	0.11 ± 0.34	0.18 ± 0.25*	0.25 ± 0.36*	0.19 ± 0.49	0.90 ± 1.52
CB	4.20 ± 0.67	0.06 ± 0.45	0.15 ± 0.39	0.26 ± 0.49	0.32 ± 0.36*	0.95 ± 1.86
HDL-C						
BB	1.18 ± 030	-0.006 ± 0.08^{b}	0.2 ± 0.1	0.01 ± 0.1	0.05 ± 0.1	0.05 ± 0.41
LB	1.18 ± 0.29	-0.003 ± 0.08^{ab}	-0.002 ± 0.07	-0.02 ± 0.07	0.02 ± 0.11	-0.01 ± 0.34
CB	1.22 ± 0.33	0.04 ± 0.07^{a}	-0.001 ± 0.09	0.03 ± 0.08	0.06 ± 0.05*	0.16 ± 0.31
LDL-C						
BB	2.41 ± 0.46	-0.22 ± 0.28*	-0.27 ± 0.26^{b*}	-0.18 ± 0.32^{b*}	0.03 ± 0.35	-0.84 ± 1.08^{b}
LB	2.33 ± 0.45	-0.17 ± 0.33	-0.23 ± 0.33^{b*}	0.04 ± 0.39^{ab}	0.08 ± 0.49	-0.39 ± 1.59^{ab}
CB	2.51 ± 0.53	-0.09 ± 0.44	0.021 ± 0.34^{a}	0.12 ± 0.45^{a}	0.21 ± 0.36*	0.28 ± 1.74^{a}

TC: HDL ratio						[1]
BB	3.58 ± 0.73	0.07 ± 0.33	0.13 ± 0.37	0.14 ± 0.29	-0.04 ± 0.53	0.39 ± 1.25
LB	3.58 ± 0.88	0.11± 0.31	0.19 ± 0.31*	0.26 ± 0.38*	0.06 ± 0.34	0.79 ± 1.31
CB	3.56 ± 0.78	0.01 ± 0.54	0.15± 0.38	0.2 ± 0.39	0.15 ± 0.37	0.6 ± 1.97

Data presented as mean ± standard deviation. *Significant change from baseline, $p<0.05$. Values in the same column marked with different letters are significantly different, $p<0.05$. TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TC:HDL ratio, total cholesterol-to-HDL ratio; AUC, Area under the curve; BB, Butter biscuit; CB, Coconut oil biscuit; LB, Lard biscuit.

Figure 4.1.

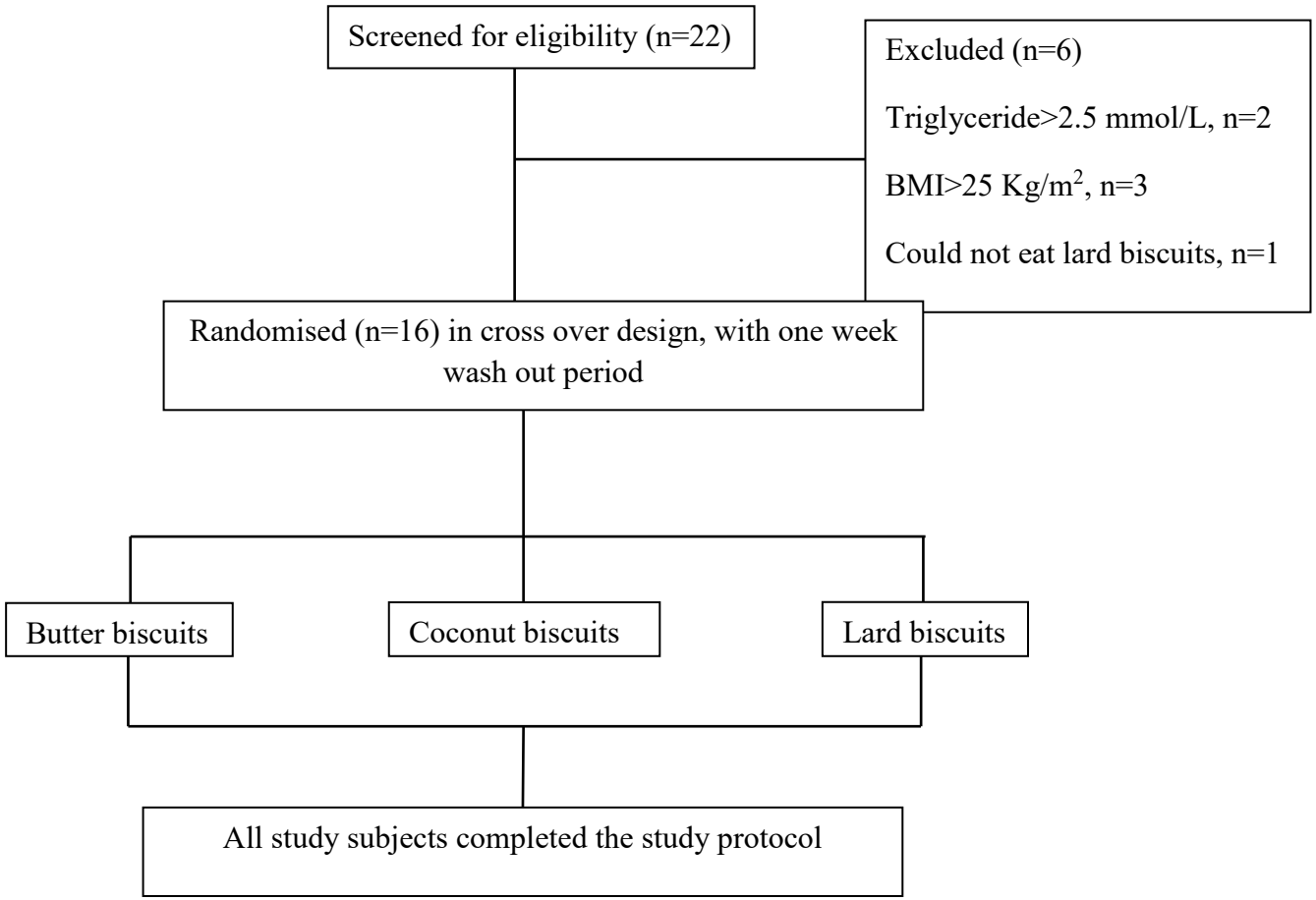
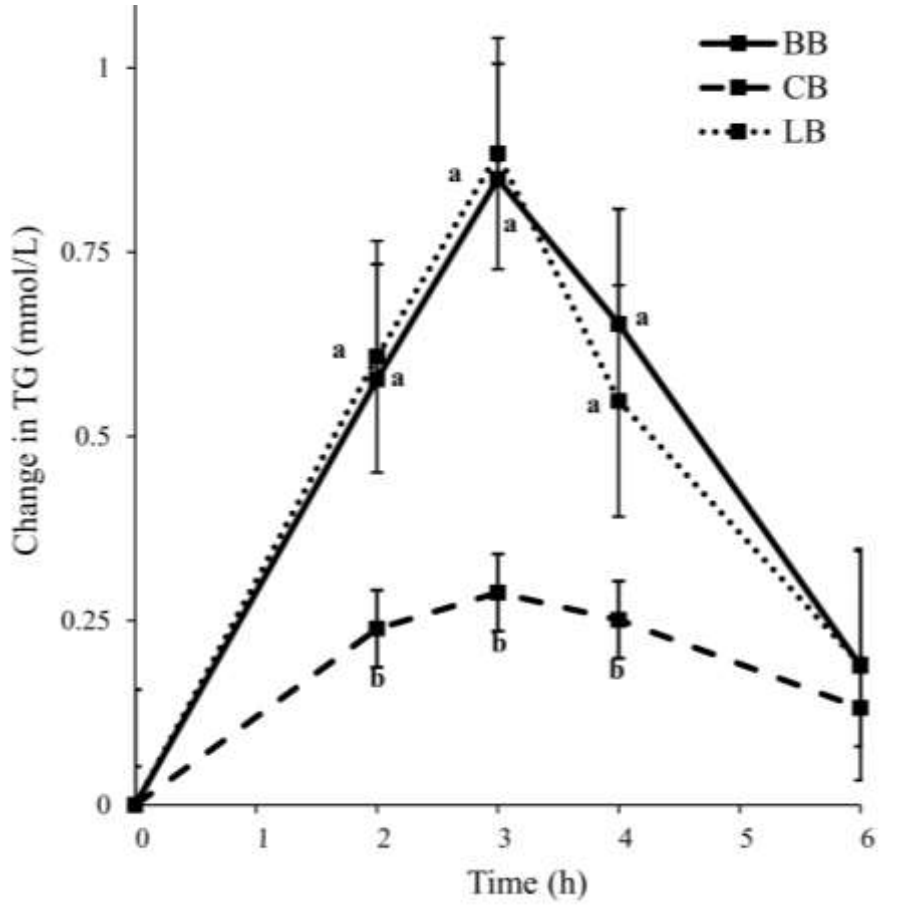


Figure 4.1: Representation of participant recruitment, screening and assessment.

Figure 4.2.

A


BB
CB
LB
Change in TG (mmol/L)
Time (h)
0
0.25
0.5
0.75
1
0
1
2
3
4
5
6
a
a
a
a
a
a
b
b
b

B

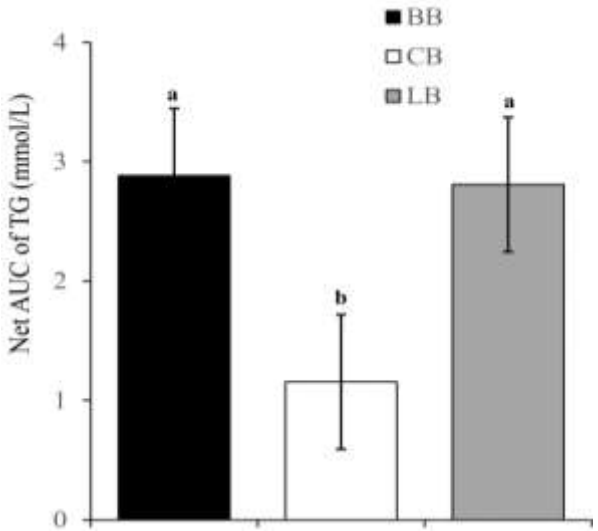

BB
CB
LB
Net AUC of TG (mmol/L)
0
1
2
3
4
a
b
a

Figure 4.2: Postprandial TG levels (A) over 6 hours in response to the ingestion of the BB, CB or LB. Values are mean ± standard error of mean (SE). Different lower-case letters represent significant difference between foods at each time point, $p<0.05$. (B) Net AUC for change from baseline in triglyceride levels (mean ± SE) for BB, CB or LB. Values in the bar diagram with different lowercase letters are significantly different, $p<0.05$. TG, triglycerides; BB, Butter biscuit; CB, Coconut oil biscuit; LB, Lard biscuit; AUC, Area under the curve

Figure 4.3.

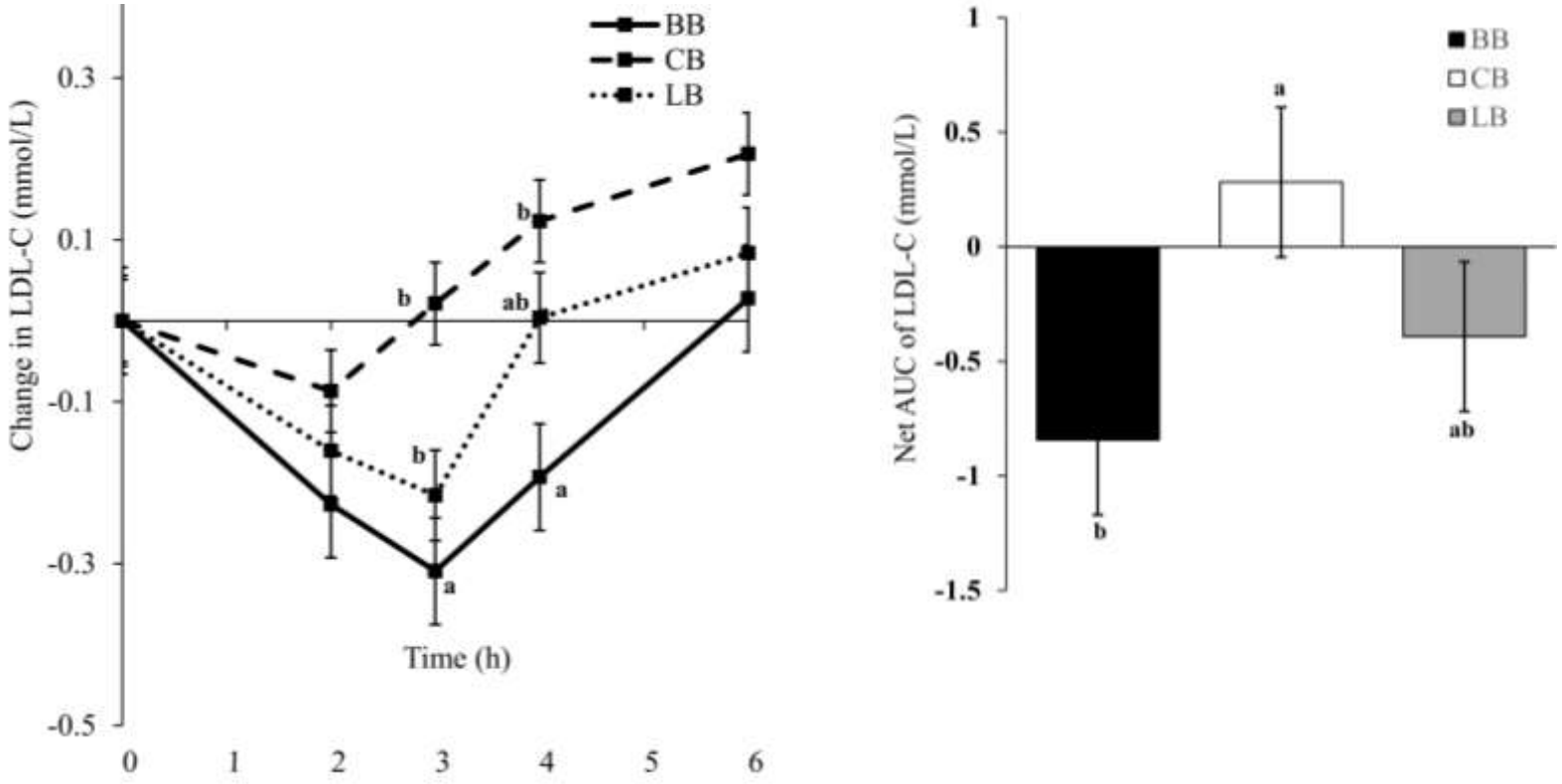


Figure 4.3: Postprandial LDL-C levels (A) over 6 hours in response to the consumption of the BB, CB or LB. Values are mean ± standard error of mean (SE). Different lower-case letters represent significant difference between foods at each time point, $p<0.05$. (B) Net AUC for change from baseline in LDL-C levels (mean ± SE) for BB, CB or LB. Values in the bar diagram with different lowercase letters are significantly different, $p<0.05$. LDL-C; low density lipoprotein; BB, Butter biscuit; CB, Coconut oil biscuit; LB, Lard biscuit; AUC, Area under the curve.

4.4. Discussion:

SFA of different chain lengths differentially modulate the postprandial lipid profile. Consumption of biscuits prepared with coconut oil rich in MCFAS resulted in reduced postprandial lipemia in comparison to biscuits containing butter (SCFA) or lard (LCSFAS). The magnitude and duration of the postprandial lipemia following meal consumption has emerged as a clinically relevant cardio-metabolic disease risk factor (214). The results presented in this study suggest that dietary MCFAS, despite being classified as saturated fatty acids, elicit lower postprandial triglyceride responses. Therefore, the role of MCFAS in promoting improved cardiovascular health warrants further investigation. Butter, the richest dietary source of SCFA, contained only 3-4% of the total fatty acids as SCFA and therefore, the amount of SCFA in butter biscuits may not have been sufficient to demonstrate a difference in postprandial lipemic response when compared to lard. Thus, suggesting that postprandial triglyceride responses to dietary SFA were driven by the predominant fatty acid present in the test biscuits.

A large majority of the studies on dietary fats have compared the postprandial lipemic responses of saturated MCFAS with unsaturated (MUFA and n-6PUFA) fatty acids (32-35). Only one study compared postprandial lipid responses to MCFAS and LCSFAS and in support of our results, demonstrated lower postprandial lipemia following MCFAS ingestion (coconut oil) compared to a LCSFAS (cocoa butter) rich meal in healthy subjects (125). Epidemiological studies of indigenous populations (India, Sri Lanka, the Philippines, Polynesia, and Melanesia) consuming high amounts of coconut products in their diet demonstrated low rates of CVD than those who consume a more western style diet (215, 216). The results of this study are also in line with those of previous studies reporting that MCFAS supplied as a single meal are transported to the liver directly via the hepatic portal circulation with very little incorporation into triglyceride packaged within chylomicrons (123, 217). Preclinical studies in swine have shown that short-term (1 week) MCFAS feeding decreased enterocyte gene expression of apolipoprotein B-48, a key transporter of dietary lipids from the intestine to the systemic circulation (218), consequently down-regulating the production of triglyceride-rich atherogenic chylomicrons (219). In addition, MCFAS undergo rapid oxidation (220), which would then result in decreased hepatic triglyceride synthesis, secretion of VLDL, and reduced postprandial plasma triglyceride levels. Higher lipoprotein lipase activity following MCFAS consumption may also contribute to increased clearance of triglyceride rich

lipoproteins from the circulation (38) resulting in overall reduced postprandial lipemia (76). In line with this rapid clearance of TG, data presented in this study suggested that TG levels, although dropping back to baseline values following MCFAS consumption, were still elevated 6 hours after LCSFAS administration. Plasma triglyceride levels peaked at 3 hours in all the three saturated fat groups, ruling out the possibility of a delayed appearance of chylomicrons into the circulation and making the delayed absorption of the MCFAS also less likely (76, 221). Therefore, a combination of lower postprandial excursion of triglyceride, causing reduced chylomicron and VLDL secretion from the duodenum and liver respectively, along with increased lipoprotein lipase activity appears to be responsible for the lower postprandial triglyceridemia following MCFAS consumption compared with LCSFAS.

The present findings contrast with studies reporting higher postprandial triglyceridemia following short-term (6 days) MCFAS consumption compared to n-6PUFA (soybean oil) (117). The conflicting postprandial triglyceride response between the current investigation and the study by Hill *et al.* may be explained by the amount of MCFAS consumed, in their study healthy male subjects consumed MCFAS in excess (150%) of their maintenance energy requirements (117). When supplied as excess calories, surplus MCFAS are preferably elongated (222) to LCSFAS and transported to adipose tissue for storage. It is apparent from these observations that overall caloric intake may also be an important determinant of the lipemic response to MCFAS.

Longer-term interventional studies in healthy and normal weight adult subjects have demonstrated lower fasting plasma triglyceride levels following MCFAS compared to LCSFAS consumption (169, 177). An inverse relationship between dietary MCFAS intake and fasting plasma triglyceride levels has also been reported in two observational studies (223, 224). Single oral feeding of MCFAS (medium chain triglyceride – MCT oil) rich meal also caused lower postprandial triglyceride levels compared to n-6PUFA (corn oil) (141) or MUFA (canola oil) (225) rich meals in healthy and hypertriglyceridemic men and women. Therefore, consumption of MCFAS appears to result in reduced triglyceridemia in comparison to LCSFAS, MUFA and n-6PUFA.

Coconut oil consumption increased postprandial LDL-C compared to butter and lard, *in* contrasts with previous studies, in which MCFAS (coconut oil) and LCSFAS (cocoa butter) did not exert differential effects on postprandial LDL-C levels (125).

Postprandially, increase in triglyceride-enriched LDL particles as a result of cholesteryl ester transfer protein-mediated exchange with triglyceride rich lipoproteins (chylomicrons and VLDL), are cleared more rapidly from the circulation (226, 227). This suggests that an increase in chylomicrons and VLDL postprandially drive a decrease in LDL particles (228, 229). In line with the scientific evidence, the greater postprandial increase in TG after lard and butter consumption decreased LDL-C, while the lower increase in TG after the consumption of the coconut oil caused an increase in LDL-C. Longer term intervention studies (4-8 weeks), however, have demonstrated lower fasting LDL-C concentrations following a coconut oil rich diet (169, 230) suggesting that postprandial increase in LDL-C following coconut oil consumption may be only transitional pending the clearance of TG-rich lipoproteins.

The postprandial TG reductions reported in the current study are of a clinically significant magnitude. A small (1 mmol/L) increase in non-fasting triglyceride levels has been associated with a five-fold increase in myocardial infarction, a three-fold increase in ischaemic stroke and a two-fold increase in cardiovascular related deaths (231, 232). Study have shown that lowering the level of TG can bring additional benefit and reduces CVD risk (233). In the present study, the magnitude of the increase in TG following coconut oil consumption was considerably lower compared to butter or lard. The choice of fatty acid chain length may therefore result in a clinically relevant difference in cardiovascular disease risk. The long-term health effects of consuming diets rich in SFA of varying lengths, and their dose-dependent responses, warrant further investigation. Whether the consumption of excess calories could increase fatty acid elongation in the liver and counter act the TG-lowering effects of MCFAS is also worthy of further investigation. A differential effect of SCFA was not apparent in this study as butter contains only 3-4% SCFA and much higher proportion of LCFAs than lard. Investigation of SCFA at higher concentration is important, particularly as there is evidence they act as signalling molecules in cellular processes that influence insulin resistance and metabolic syndrome (234).

In conclusion, consumption of coconut oil caused lower postprandial triglyceridemia compared to butter and lard. Although research into the clinical significance of dietary MCFAS is in its preliminary stages, outcomes from this study suggest that consumption of MCFAS compared to LCSFAS may have distinct effects on postprandial lipid

metabolism. Dietary recommendations to lower the saturated fat intake may not be applicable to foods containing medium chain fatty acids.

Chapter 5:

General Discussion and Conclusions

5.1. Key findings

SFA of different chain lengths displays different metabolic properties and functions, therefore SFA should not be considered as a single group in terms of its role in health and disease. Fatty acids are rarely consumed in isolation, however, majority of the experimental research on dietary fats to date is conducted considering the impact of single SFA on blood lipids and other CVD biomarkers. There is limited data directly comparing blood lipids with individual effects of MCFAS versus LCSFAS in humans. To date, the differential effect of chain length of SFA (SCFA, MCFAS, or LCSFAS) on postprandial blood lipids has not been directly compared. It has been previously demonstrated that diet rich in MCFAS are not incorporated into chylomicron and therefore may influence blood lipid profile. A large body of literature suggests the advantages of MCT oil compared to long-chain SFA on blood lipid profiles and CVD health benefits.

Omega-3 fatty acid found abundantly in fish are found to lower fasting as well postprandial TG concentration, suggesting that omega-3 fatty acid may have beneficial effect in the management of cardiovascular disease (235). Therefore, people who took fish oil supplements were excluded from the clinical trial (1). Reduction of postprandial lipemia would be seen in participants currently being treated with lipid lowering agents (236); therefore, participants on lipid-lowering medications were excluded from the clinical trial. Since decisions made in selection of exclusion criteria applied in crossover trial could have a substantial effect on trial results, careful consideration was made when making decision.

This thesis has examined the differential effect whereby MCFAS influence the blood lipid response differentially. The publication presented in Chapter 3, demonstrated for the first time the differential effects of MCFAS and LCSFAS on blood lipids and apolipoprotein in healthy males and females. Specifically, the study demonstrated the efficacy of MCFAS consumption to increase HDL-C levels and apoA-I levels compared to LCSFAS. To address our hypothesis, an acute study was conducted to determine the short-term effects of feeding biscuits rich in SCFA, MCFAS, or LCSFAS on the post-prandial blood lipids (total, LDL and HDL cholesterol and triglycerides). An attenuated postprandial lipaemic response following MCFAS (coconut oil) compared with SCFA (butter) or LCSFAS (lard) provided evidence regarding the differential impact of the chain length of SFA on the postprandial lipid response. However, butter did not differentially impact the

overall triglyceride response compared with lard; which suggest that LCSFAS in the butter may have negative effects and the amount of SCFA in the butter may not be enough (3% butyric acid) to counter-balance the effect of LCSFAS. Previous studies have compared postprandial TG response of dietary MCFAS and unsaturated fats, and demonstaretd lower postprandial TG response compared to n-6PUFA, n-3PUFA and MUFA. Only one study compared postprandial TG response within SFA and demonstrated lower postprandial lipemia following MCFAS (coconut oil) compared to LCSFAS (cocoa butter). However, an analysis of post-prandial lipid levels following whole food sources of SCFA, MCFAS, or LCSFAS had not been examined before.

At the present time, research regarding health effects of saturated fats consumed in the diet is not clear. Collectively, the findings from these research studies incorporating systematic review/meta-analysis and an acute study provide a plausible explanation for the variable results in the literature in the lipid modulating response with MCFAS supplementation. Analysis of dose-response effect of MCFAS intake on change in HDL-C concentration has shown HDL-C elevation with a threshold at about 30gday approximately. In human, diet rich in coconut oil will certainly result in a measurable increase in HDL and may account for the protective effect against CVD. Further research on the topic is required to make a recommendation on amount of cocnut oil to be consumed on daily basis for health benefits. In summary, this study highlights that the food source of SFA modulates its effect on postprandial lipemia.

5.2. Strength and limitation of the review

Strength and limitations related to the individual research aim, and study designs were discussed in chapter 3 and 4. The inconsistency in the available SFA and blood lipids literature has been compounded by the fact that SFA were considered as a single group which preclude definite conclusions. Most importantly, the systematic review and meta-analysis has been the first meta analysis directly comparing the blood lipid response following MCFAS vs. LCSFAS rich diet. Study 2 employed a single-blinded randomized crossover trial; therefore, the effect of inter-individual variability was minimized by directly comparing the postprandial blood lipid from the same participant supplemented with SCFA vs. MCFAS vs. LCSFAS. In addition, the test meals comprised of commonly consumed food products making it more clinically relevant as people do not consume

individual fats on their own. However, a potential limitation of the study was the fact that the amount of SCFA in butter biscuit was potentially insufficient to counterbalance the amount of LCSFAS. Therefore, the amount of short chain fatty acid may confound the interpretation of non-significant findings, making interpretation of the effects of SCFA difficult. Also, a sufficient amount of SCFA in the intervention might have made a difference to the effect of SCFA on postprandial lipemia. It should be acknowledged that this study specifically assessed acute and not chronic responses and it may be that long-ter consumption is required to detect any differences in metabolic responses. It is also important to note that when consumed in excess calories, surplus MCFAS are preferably elongated to LCSFAS and transported to adipose tissue for storage. Therefore, overall energy intake is an important determinant of the lipemic effects of short and medium chain SFA.

5.3. Impact on nutrition research

The effect of different chain-length fatty acids on metabolism is unclear in the literature. Not all saturated fats are the same, and the cardiovascular health of the population would benefit from greater understanding of the differential effects of healthy versus unhealthy saturated fats. The study hypothesises that short and medium chain saturated fat do not adversely affect lipid metabolism. This research may aid in the design of dietary strategies for cardiovascular risk reduction and contribute to revised dietary guidelines for healthy eating.

5.4. Conclusion

The work embodied in this thesis provides substantial evidence that medium-chain SFA differentially influence blood lipids compared to long-chain SFA and validate the research hypothesis that forms this thesis. Our observation from a systematic review indicates that diets rich in MCFAS are effective in increasing HDL-C level compared to diet rich in LCSFAS, however further studies are needed to establish the possible mechanism involved. Most studies (10 out of 12) included in the meta-analysis were done many years ago (20 years); therefore, further research may therefore be of interest to establish the beneficial effect of MCFAS. From our acute study, we were able to conclude

that coconut oil lowers postprandial lipemia compared to butter and lard. Although, the findings from intervention study did not confirm the findings from meta-analysis, MCFAS when substituted for LCSFAS in isocaloric diets and not consumed as excess energy, may improve heart health. It seems that under the conditions that we used (butter, 3% SCFA), amount of SCFA was not enough to observe its beneficial effect on postprandial lipemia. Since a substantial amount of SCFA doesn't exist in single food, the small proportion of SCFA present in butter may confound the interpretation of non-significant findings, making interpretation of the effects of SCFA difficult from this study. The research reported in this thesis provides important insights and explains the controversies surrounding the health effects of SFA. The result of this study adds valuable insight into the beneficial effect of MCFAS in the diet; and it is important that dietary guidelines consider the impact of whole food containing SFA rather than individual SFA in the diet while developing strategies that aim to improve overall diet quality. Therefore, consideration of the whole food source from which dietary fats are derived may impact health and disease. It becomes evident when considering these findings that not all saturated fatty acids have the same effects on human health; therefore, a stronger focus on foods containing predominant SFA-specific health effects is needed, at least within the scientific and medical community.

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Appendices

Appendix 1

Statements from 4 co-authors relating to paper published with Nisha Panth (Chapter 3)

- Panth N, Abbott KAA, Dias C, Wynne Katie, Garg ML. Differential effects of medium and long chain saturated fatty acids on blood lipid profile: a systematic review and meta-analysis; The American Journal of Clinical Nutrition. 2018; 108(4): 675-687. DOI: 10.1093/ajcn/nqy167.

Statement from co-authors relating to a paper published with Nisha Panth

I confirm that Nisha Panth contributed to the following publication:

- Panth N, Abbott KAA, Dias C, Wynne Katie, Garg ML. Differential effects of medium and long chain saturated fatty acids on blood lipid profile: a systematic review and meta-analysis; The American Journal of Clinical Nutrition. 2018; 108(4): 675-687. DOI: 10.1093/ajcn/nqy167.

Nisha Panth contributed to study conception and design; analysed the data; and wrote the manuscript. Nisha Panth and Kylie Abbott conducted the study. Kylie Abbott, Cintia Dias, Manohar Garg, Katie Wynne contributed to study conception. All authors read and approved the final manuscript.

_____ Date: 2/11/2018

Ms Kylie Abbott

_____ Date: 2/11/2018

Dr. Cintia Dias

_____ Date: _____

Dr Katie Wynne

Date: 9/11/18

Professor Manohar Garg

_____ _____ Date: November 01, 2018

(Signature of Assistant Dean Research Training)

Appendix 2

Statements from 4 co-authors relating to papers published with Nisha Panth (Chapter 4)

- Panth N, Dias C, Singh H, Wynne Katie, Garg ML. Medium chain fatty acids lower postprandial lipemia: a randomized crossover trial. 2018; submitted to Clinical Nutrition.

Statement from co-authors relating to a paper published with Nisha Panth

I confirm that Nisha Panth contributed to the following publication:

- Panth N, Dias C, Singh H, Wynne Katie, Garg ML. Medium chain fatty acids lower postprandial lipemia: a randomized crossover trial. 2018; submitted to Clinical Nutrition

Nisha Panth contributed to study conception and design, conducted the study; recruited subjects; collected and analysed data. Nisha Panth and Manohar Garg wrote the manuscript. Cintia Dias, Manohar Garg, Katie Wynne contributed to study conception, design and coordination. All authors read and approved the final manuscript.

_____ Date: 2/11/2018

Dr. Cintia Dias

Date: 9/11/18

Dr. [redacted]atie Wynne

Date: 2 November 2018

Professor Harjinder Singh

_____ Date: _____

Professor Manohar Garg

Date: November 01, 2018

(Signature of Assistant Dean Research Training)

FLYER

CAN SATURATED FAT BE GOOD FOR HEART HEALTH?

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

RESEARCH STUDY

www.newcastle.edu.au

Researchers at the University of Newcastle would like to find out the differential effect of short chain fats (butter), medium chain fats (coconut oil) and long chain fats (lard) on blood lipids and other cardiovascular risk factors in healthy participants.

Are you aged 18-65 years with BMI 18.5 – 24.9 Kg/m^2

Project supervisors

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Nutraceuticals Research Group

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For further information, please contact:

Nisha Panth on Phone: +(02) 4921 5638 or Email: nisha.panth@uon.edu.au

Complaints about this research **Version 1.2 Last updated 27/02/2017**

This project has been approved by the University of Newcastle Human Research Ethics Committee, Approval No H-2016-0429. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 230, Australia, Telephone (02) 49216333, email: Human-Ethics@newcastle.edu.au

Appendix 4

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INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

Document Version 1.1; dated 30/11/2016

Differential effects of short, medium and long chain saturated fatty acids on cardiovascular risk factors.

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.
Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ days per week

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ hours per day
_____ minutes per da

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ days per week

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ hours per day
_____ minutes per day

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time
as part of your work? Please do not count any walking you did to travel to or from work.

_____ days per week

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ hours per day
_____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle**

like a train, bus, car, or tram?

_____ days per week

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ hours per day
_____ minutes per day

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ days per week

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ hours per day
_____ minutes per day

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ days per week

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ hours per day
_____ minutes per day

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time.

During the **last 7 days**, on how many days did you do **vigorous** physical activities like
heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ days per week

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ hours per day
_____ minutes per day

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ days per week

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ hours per day
_____ minutes per day

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ days per week

☐ No moderate activity inside home → ***Skip to PART 4:***

RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ hours per day
_____ minutes per day

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ days per week

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ hours per day
_____ minutes per day

22. Think about only those physical activities that you did for at least 10 minutes at a time.

During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ days per week

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ hours per day
_____ minutes per day

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ days per week

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ hours per day
_____ minutes per day

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ hours per day
_____ minutes per day

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ hours per day
_____ minutes per day

This is the end of the questionnaire, thank you for participating.

Reference: https://sites.google.com/site/theipaq/questionnaire_link

Appendix 5

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EATING ATTITUDE TEST

Document Version 1.1; dated 30/11/2016

Differential effects of short, medium and long chain saturated fatty acids on cardiovascular risk factors.

Instructions: This is a screening measure to help you determine whether you might have an eating disorder that needs professional attention. This screening measure is not designed to make a diagnosis of an eating disorder or take the place of a professional consultation. Please fill out the below form as accurately, honestly and completely as possible. There are no right or wrong answers. All of your responses are confidential.

Part A: Complete the following questions:

1) Birth Date	Month:	Day:	Year:	2) Gender:	Male ☐ Female ☐
3) Height	Feet :	Inches:			
4) Current Weight (lbs.):		5) Highest Weight (excluding pregnancy):			
6) Lowest Adult Weight:		7: Ideal Weight:			

Part B: Check a response for each of the following statements:		Always	Usually	Often	Some times	Rarely	Never
1.	Am terrified about being overweight.	☐	☐	☐	☐	☐	☐
2.	Avoid eating when I am hungry.	☐	☐	☐	☐	☐	☐
3.	Find myself preoccupied with food.	☐	☐	☐	☐	☐	☐
4.	Have gone on eating binges where I feel that I may not be able to stop.	☐	☐	☐	☐	☐	☐
5.	Cut my food into small pieces.	☐	☐	☐	☐	☐	☐
6.	Aware of the calorie content of foods that I eat.	☐	☐	☐	☐	☐	☐
7.	Particularly avoid food with a high carbohydrate content (i.e. bread, rice, potatoes, etc.)	☐	☐	☐	☐	☐	☐
8.	Feel that others would prefer if I ate more.	☐	☐	☐	☐	☐	☐
9.	Vomit after I have eaten.	☐	☐	☐	☐	☐	☐
10.	Feel extremely guilty after eating.	☐	☐	☐	☐	☐	☐
11.	Am preoccupied with a desire to be thinner.	☐	☐	☐	☐	☐	☐
12.	Think about burning up calories when I exercise.	☐	☐	☐	☐	☐	☐
13.	Other people think that I am too thin.	☐	☐	☐	☐	☐	☐
14.	Am preoccupied with the thought of having fat on my body.	☐	☐	☐	☐	☐	☐
15.	Take longer than others to eat my meals.	☐	☐	☐	☐	☐	☐
16.	Avoid foods with sugar in them.	☐	☐	☐	☐	☐	☐
17.	Eat diet foods.	☐	☐	☐	☐	☐	☐
18.	Feel that food controls my life.	☐	☐	☐	☐	☐	☐
19.	Display self-control around food.	☐	☐	☐	☐	☐	☐
20.	Feel that others pressure me to eat.	☐	☐	☐	☐	☐	☐
21.	Give too much time and thought to food.	☐	☐	☐	☐	☐	☐
22.	Feel uncomfortable after eating sweets.	☐	☐	☐	☐	☐	☐
23.	Engage in dieting behavior.	☐	☐	☐	☐	☐	☐
24.	Like my stomach to be empty.	☐	☐	☐	☐	☐	☐
25.	Have the impulse to vomit after meals.	☐	☐	☐	☐	☐	☐
26.	Enjoy trying new rich foods.	☐	☐	☐	☐	☐	☐

Part C: Behavioral Questions: In the past 6 months have you:		Never	Once a month or less	2-3 times a month	Once a week	2-6 times a week	Once a day or more
A	Gone on eating binges where you feel that you may not be able to stop? *	☐	☐	☐	☐	☐	☐
B	Ever made yourself sick (vomited) to control your weight or shape?	☐	☐	☐	☐	☐	☐
C	Ever used laxatives, diet pills or diuretics (water pills) to control your weight or shape?	☐	☐	☐	☐	☐	☐
D	Exercised more than 60 minutes a day to lose or to control your weight?	☐	☐	☐	☐	☐	☐
E	Lost 20 pounds or more in the past 6 months	Yes ☐		No ☐			

* Defined as eating much more than most people would under the same circumstances and feeling that eating is out of control

Appendix 6

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INFORMATION STATEMENT (ACUTE STUDY)

Document Version 1.3; dated 10/03/2017

Differential effects of short, medium and long chain saturated fatty acids on cardiovascular disease risk factors.

You are invited to participate in the research project identified above which is being conducted by Ms. Nisha Panth as part of her PhD studies at the University of Newcastle under the supervision of Prof. Manohar Garg, Prof. Harjinder Singh, Dr. Katie Wynne, and Dr. Cintia Dias.

Why is the research being done?

It is well known that short (SCFA; butter) and medium (MCFAS; coconut oil) chain fatty acids are readily digested and metabolized in comparison to long chain saturated fatty acids (LCFA; lard, tallow). Evidence from animal studies have demonstrated that there is differential effect of butter, coconut oil and lard on cardiovascular health. Therefore, the purpose of the research is to find out the differential effect of butter, coconut oil and lard on blood lipids, glucose levels and endothelial function in humans.

Who can participate in the research?

We are seeking healthy normal weight range individuals with body mass index (BMI) between 18 – 25Kg/m^2, aged between 18 and 65 years old.

Unfortunately, you cannot participate if you:

- Have BMI >24.9 Kg/m^2;
- Are pregnant, planning to become pregnant or breast feeding;
- Taking lipid-lowering drugs (e.g. statins) or supplements (e.g. fish oil), or non-steroidal anti-inflammatory drugs (NSAIDs), or anti-hypertensive drugs, or vasodilators, or antioxidants vitamins.
- Are dieting or have any eating disorders;
- Have sensitivity or intolerance to any of the food products used in the study;
- Have history of congestive heart failure, stroke, myocardial infarction, coronary artery bypass graft, or atherosclerotic CVD, or pulmonary disease;
- Have history of diabetes, hypertension, triglycerides higher than 3.0 mmol/L; total cholesterol higher than 5.5 mmol/L;
- Have history of gastrointestinal disorder, gall bladder disease, or liver disease;
- Smoke
- Are vegetarian or vegan

What would you be asked to do?

If you agree to participate, you will be asked to attend Nutraceuticals Research Program (Level 3 of the Medical Science Building, University of Newcastle, Callaghan campus) after an overnight fast (water allowed) on 3 separate occasions. You will be guided to consume a standard low fat meal, the evening before the intervention. You should refrain from alcohol and exercise for 24 hours prior to intervention.

During each visit, you will be asked to do the following:

- Complete a brief medical questionnaire;
- Complete a brief physical activity questionnaire;
- Complete Appetite Sensation at 0 hour and every 2 hours after test food consumption;
- Complete a 24 hr food recall;
- Have your blood pressure, height, weight, waist circumference and body composition (% body fat and lean mass) measured. Body composition will be measured with the subject in bare foot and light clothes.

- Complete eating attitudes test;
- Consume the test foods (sweet biscuits);
- Donate blood samples by finger prick before and after (0, 2, 3, 4 and 6 hour) food consumption;

You will be randomly allocated to consume 6 small biscuits prepared with 40g of either butter, or coconut oil, or lard. You will be required to consume all 3 test foods in random order with at least one week gap in between the test days.

What choice do you have?

Participation in this research is entirely your choice. Only those people who give their informed consent will be included in the project. If you do decide to participate, you may withdraw from the project at any time without giving a reason.

How much time it will take?

You will need to visit University of Newcastle three days in total. Each day should take 6-7 hours of your time.

What are the risks and benefits of participating?

Blood will be collected from the middle and ring finger of the non-dominant hand using capillary tubes. A slight pinch may be experienced as blood is being taken. There is a slight risk of bleeding from the collection site, bruising and pain.

Should the information you provide in questionnaires or the results of your blood tests indicate potential health issues, you will be referred to your general practitioner (GP) and this may affect your continuation of participating in the study.

Participants will acquire knowledge and contribute to the scientific advancements of nutrition in the prevention of chronic diseases. You will receive your individual blood test results in the e-mail and in addition, overall results of this research will be made available to you on request.

If you choose to participate, you will receive $20 per visit to compensate for travel expenses.

How will your privacy be protected?

All anthropometric and body composition measurements will be performed in a separate room one person at a time for privacy reasons. Your information will be treated with the same respect for privacy and confidentiality as is undertaken for all medical information collected about you during your visits to your local doctor. Access to the collected information will be limited to the named investigators only. Non-identifiable data may be also be shared with other parties to encourage scientific scrutiny, and to contribute to further research and public knowledge. All collected information directly from the participants will be storage in the original paper copy in a locked filing cabinet at the Medical Science Building at the University of Newcastle accessible to the investigators. All data will only be identifiable by a participant identification number with no other identifying details on file. During statistical data analysis, the database will be stored in a password protected computer file.

How will the information collected be used?

Results of this research will be presented at the national and international conferences, published in scientific journals and will be published in Nisha Panth's PhD thesis. Individual participants will not be identified in any report arising from the project. Blood samples will be destroyed once the analysis of data has been completed and all publications/reports and thesis accepted.

What do you need to do to participate?

Please read this Information Statement and be sure you understand its contents before you consent to participating. If there is anything you do not understand, or you have questions please contact the study principle investigator. If you would like to participate, please complete the attached health questionnaire and Consent Form and return them in the envelope provided to the investigators. The research team will contact you to make your first appointment.

At the conclusion of this study, you may be contacted for participation in a longer-term feeding trial involving these biscuits, however you are in no way obligated to participate.

Further information

If you would like further information regarding this study please contact Professor Manohar Garg, or Ms Nisha Panth (4921 5638), **Email:** nisha.panth@uon.edu.au

Thank you for considering this invitation to participate in research project.

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Complaints about this research

This project has been approved by the University of Newcastle Human Research Ethics Committee, Approval No H-2016-0429. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, Telephone (02) 4921 6333, Email: Human-Ethics@newcastle.edu.
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Appendix 7

Professor Manohar Garg
Nutraceuticals Research Group
School of Biomedical Sciences and Pharmacy
Telephone (02) 4921 5647

CONSENT (ACUTE STUDY)

Document Version 1.3; dated 10/03/2017

Differential effects of short, medium and long chain saturated fatty acids on cardiovascular disease risk factors

I give my consent to participate in the research project designed to test the concept that short, medium and long chain saturated fatty acids have differential effects on cardiovascular risk factors. I have been provided the Information Sheet, a copy of which I have retained.

- ✓ I agree to attend Nutraceuticals Research Program (Level 3 of the Medical Science Building, University of Newcastle, Callaghan campus) with an overnight 10 hours fast (except water) on three occasions. Each test day I need to be available for up to 6-7 hours.
- ✓ I understand that my participation is **voluntary** and entirely my choice.
- ✓ I agree to provide my GP contact details.
- ✓ I understand I can **withdraw** my consent for participation at any time, I **do not** have to give any **reason** and I have the option of withdrawing any data which identifies me.
- ✓ I understand that I will need to consume a standard meal the day before the experiment and refrain from strenuous exercise and alcohol consumption 24 hours prior to my appointment.
- ✓ I understand that I will be randomly allocated to biscuits prepared with 40 g of fat in the form of either butter, or coconut oil, or lard. I will be required to consume all 3 test foods in random order with at least one-week gap between test days.
- ✓ I understand that at each visit, I will be required to have my (blood pressure, height, weight, body composition) measured; complete (a brief medical, brief physical activity, eating attitudes test, appetite sensation) questionnaire and complete a 24-hour food diary.
- ✓ I understand that I need to donate 5 blood samples by finger prick. Blood will be collected before and (2, 3, 4 and 6 hours) after test food consumption.
- ✓ I understand that I will go for ultrasound of brachial artery before and 3 hours after test food consumption.
- ✓ Feedback of results from the investigation and other information regarding to the outcome of the research can be made available to me on request at the completion of the study.

- ✓ I will receive my individual blood test results in the e-mail and in addition, overall results of this research will be made available to me on request.
- ✓ I understand that my personal information will remain **confidential** to the researchers.

Signature:_____ Date:_____

Full name printed: _____

Email: _____

Appendix 8

Professor Manohar Garg
Nutraceuticals Research Group
School of Biomedical Sciences and Pharmacy
Telephone (02) 4921 5647

PARTICIPANT ASSESSMENT CRITERIA

"Version 1.1; dated 30/04/2012"

Participant ID Number: _____Trial Day: _____ Date: ___/____/___

Inclusion Criteria (must be either one)

- ☐ Healthy male aged 18 – 65 years (DOB:__/__/____)
- ☐ Healthy female aged 18 – 65 years (DOB:__/__/____)

Exclusion Criteria

✓	Currently on cholesterol lowering drugs or fish oil supplements	Yes No
✓	Current regular consumption of 2 or more fish meals a week	Yes No
✓	History of congestive heart failure, stroke, myocardial infarction, coronary artery bypass graft, or atherosclerotic CVD	Yes No
✓	Diabetes	Yes No
✓	Triglycerides higher than 10 mmol/L; LDL cholesterol higher than 5 mmol/L	Yes No
✓	History of gastrointestinal disorder	Yes No
✓	History of liver disease	Yes No
✓	Current smoker	Yes No
✓	Pregnant or breast feeding women	Yes No
✓	Do you have any issues with blood tests (difficult veins, history of fainting)	Yes No

Criteria assessed by: _____ Date: ___/___/___

Reviewed by investigator: _____ Date: ___/___/___

Appendix 9

FOOD DIARY

Instructions for recording food and drink consumption (24 hours)

"Version 1.1; dated 30/04/2012"

You are asked to record everything you eat and drink for a period of 24 hours. The information you record is very important to the success of this study. These instructions will help you to fill in your food and drink consumption in the most accurate manner.

If you have any questions, please do not hesitate to contact Nisha Panth on 4921 5638.

Instructions:

- ✓ **Write down everything that you eat and drink (including water) for 24 hours.**
- ✓ **Complete the record in the time frame you are given by Nisha Panth.**
- ✓ **Use as many pages as you need (pages are provided):**
  - List the food and give a description, including method of cooking (e.g. grilled rump steak or fried rump steak). An example of how to record the information is given below.
  - State the weight of the food or use standard household measure, such as a cup, a tablespoon, a teaspoon.
  - Specify brand names of products for processed foods (e.g. Nestle diet yoghurt)
  - Include supplements, and any condiments such as sauce or salad dressing
- ✓ **Try to record each item when you eat or drink so that nothing is forgotten.**

Example:

Time/Meal (Breakfast/Lunch/Dinner)	**Food/Drink + description**	**Quantity**
7 am (Breakfast)	Kellogg's corn flakes	1 cup
	Dairy Farmer's lite milk	100 mL
	Boiled egg	2 medium
	Black coffee – Nescafe instant	1 cup
12 noon (Lunch)	Salad sandwich – wholegrain bread	2 slices
	Baby spinach leaves	1 cup
	Tomato slices	3 slices
	Edgell's tinned beetroot	30 grams
	Grated carrot	1 tablespoon
	Red delicious apple	1 large
	Nestle tropical fruit diet yoghurt	125 mL
	Water	500 mL
3pm (Dinner)	Diet coke	500 mL
	Etc.	

FOOD DIARY

Participant code: _____ Day: _____

Time/Meal	Food/Drink + description	Quantity

Appendix 10

FACULTY OF HEALTH

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

MEDICAL QUESTIONNAIRE

"Version 1.1; dated 30/04/2012"

Differential effects of short, medium and long chain saturated fatty acids on cardiovascular disease risk factors

Participant code: _____

Date of birth: ___/___/___ Sex: _____ Height: _____cm Weight: _____kg

1. Please list all current medical conditions for which you are presently receiving treatment:

Condition_____	Year diagnosed_____
Condition_____	Year diagnosed_____
Condition_____	Year diagnosed_____
Condition_____	Year diagnosed_____
Condition_____	Year diagnosed_____
Condition_____	Year diagnosed_____

2. Have you ever had:

A stroke?	Yes No	Renal disease?	Yes No
Heart attack?	Yes No	Angioplasty?	Yes No
Angina?	Yes No	By-pass surgery?	Yes No
Other heart-related problems?	Yes No	Other heart/vascular surgery?	Yes No
Hepatitis?	Yes No		

3. Please list all medications that you take as prescribed by a doctor (brand name, dose, and dosage regimen - frequency)**:**

_____

_____

_____

4. Please list all over-the-counter medications you take (brand name, dose, and dosage regimen - frequency)**:**

_____

_____

_____

5. Please list all vitamin, mineral, and/or herbal supplements you take REGULARLY (brand name, dose, and dosage regimen - frequency):

_____

_____

_____

6. How many alcoholic beverages do you normally consume per week?

0-3_____ 4-7_____ 8-10_____ More than 10_____

7. Do you smoke? Yes No

8. How often do you currently exercise, and what types of exercise do you do?

Exercise	Hours per day	Hours per week	Hours per month
Walking, medium pace			
Walking, briskly			
Running/jogging			
Bike riding			
Gym			
Sport (specify):			
Other physical activities (specify):			

Appendix 10

Search strategy in Scopus database

"coconut oil*" OR "palm kernel oil*" OR "medium chain triglyceride*" OR "medium chain fatty acid*" OR "caproic acid" OR caproates OR "hexanoic acid" OR "caprylic acid" or caprylates OR "octanoic acid" OR "capric acid" OR "decanoic acid*" OR "lauric acid*" OR monolaurin OR trilaurin

Tallow OR "beef fat" OR suet OR lard OR "palm oil" OR butter OR cheese OR "myristic acid*" OR "palmitic acid*" OR "stearic acid*"

Lipids OR lipoprotein* OR apolipoprotein* OR chylomicrons OR "blood lipid*" OR "plasma lipid*" OR triglyceride* OR hyperlipidemia* OR "Serum lipid*" OR cholesterol OR dyslipidemia* OR "LDL HDL ratio" OR triacylglycerol* OR hypercholesterolemia OR "lipid disorder*" OR hypertriglyceridemia* OR hypolipidaemia*

Random* OR trial OR groups