Modulation of VLDL triglyceride metabolism

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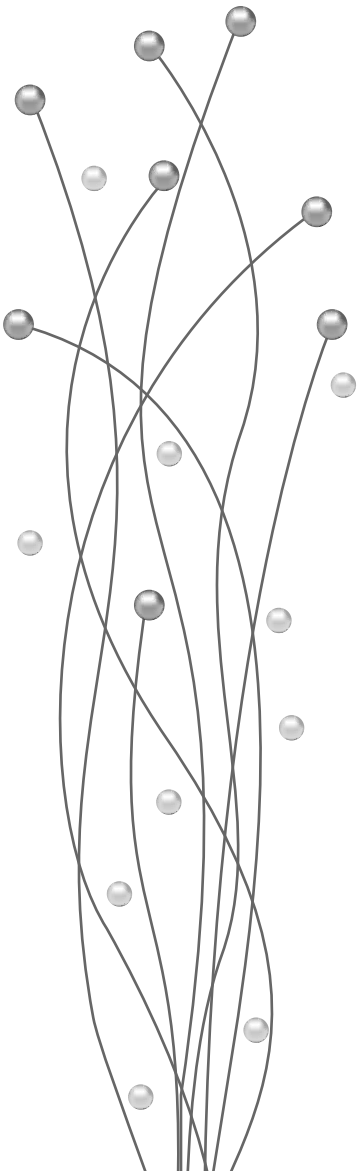
Chapter 1

General introduction



Lipids are essential for life and fulfil multiple functions in energy homeostasis and cellular biology. Two of these lipids are cholesterol, the structural component of cell membranes and steroid hormones, and triglycerides (TG), the main source of energy for both exercise and storage. Excess intake of energy increases the storage of TG and results in overweight and obesity. Since obesity is becoming ever more prevalent in our society due to a sedentary lifestyle combined with a calorie-rich Western diet, it is important to understand pathways involved in the uptake, distribution, oxidation and storage of TG.

In fact, obesity is a global epidemic as stated by the World Health Organization[1] and a major public health concern since excessive overweight is associated with various diseases such as diabetes mellitus type 2 (DM2) and cardiovascular disease (CVD)[2]. The link between TG metabolism, obesity and the development of pathology is subject to intense investigation. TG are composed of a glycerol backbone and three fatty acids (FA). Prior to transmembrane transport, TG are hydrolyzed to FA and after they have been taken up, these FA are re-esterified to TG for storage. Since free FA (FFA) are cytotoxic[3], it is thought that the (mis)handling and distribution of TG derived FA plays a central role in the pathogenesis of overweight related diseases such as DM2 and CVD[4, 5].

Lipoprotein Metabolism

The most common lipids in our diet are cholesterol and TG. Since lipids are hydrophobic, they are transported in the circulation in water-soluble spherical particles called lipoproteins. These lipoproteins carry TG and esterified cholesterol (cholesteryl esters, CE) in their core, surrounded by a shell of phospholipids, free cholesterol and proteins termed apolipoproteins (apo's). Based on their composition and origin, lipoproteins can be divided into five major classes e.g. chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL)[6]. Lipid metabolism can roughly be divided in three major pathways: the exogenous pathway important for transport of dietary lipids, the endogenous pathway important for the transport of lipids during fasting and the reverse cholesterol transport pathway important for the transport of cholesterol from tissues (Fig. 1).

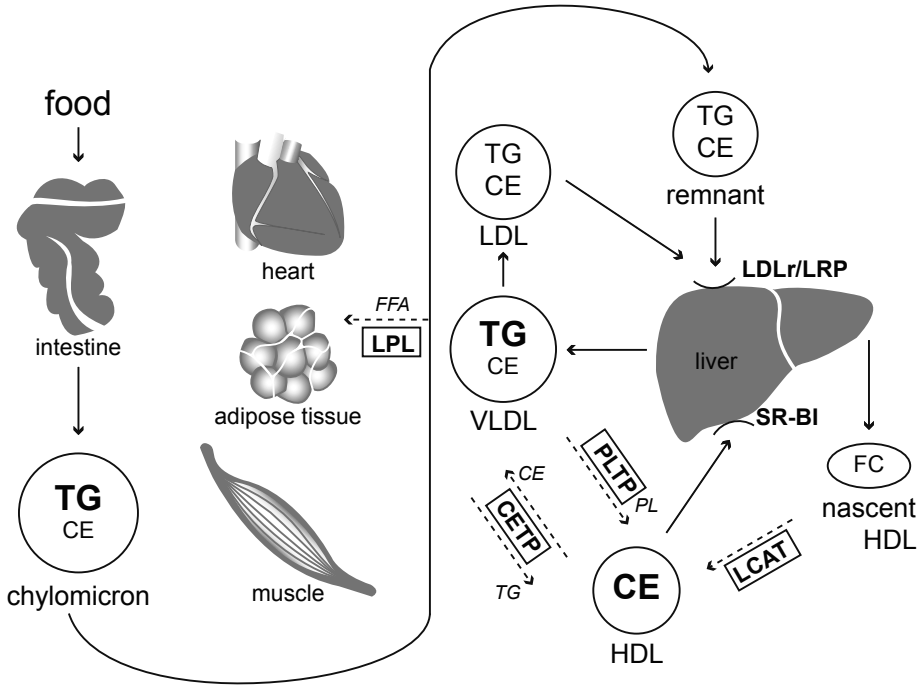


Figure 1. Schematic overview of lipoprotein metabolism.
See text for explanation. CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FC, free cholesterol; FFA, free fatty acids; LCAT, lecithin:cholesterol acyltransferase; LDLr, LDL receptor; LPL, lipoprotein lipase; LRP, LDLr related protein; PL, phospholipid; SR-BI, scavenger receptor BI; TG, triglycerides.

Exogenous pathway

In the intestine, dietary lipid is emulsified by the action of bile and TG are hydrolyzed in glycerol and FA by pancreatic lipase[7]. Cholesterol, glycerol and FA are absorbed by the intestinal cells where FA are re-esterified to TG. The TG and cholesterol are packaged in chylomicrons and secreted in the lymph from which they are transported to the blood circulation[8, 9]. Nascent chylomicrons are rich in TG but also contain phospholipids, CE and apolipoproteins apoAI, apoAIV, apoB48 and apoCs[10]. Upon entering the circulation, chylomicrons are processed by lipoprotein lipase (LPL)[8]. LPL hydrolyzes TG, thereby delivering FA to peripheral tissues were it can be used as energy source (heart and skeletal muscle) or can be stored in adipose tissue. The resulting TG depleted remnant chylomicrons are taken up by the liver, mainly via apoE specific recognition sites on the hepatocytes such as the LDL receptor (LDLr) and the LDLr related protein (LRP)[11, 12].

Endogenous pathway

Especially in the postprandial state, the liver is the main site of secretion of cholesterol and TG, packaged in VLDL for transport to peripheral tissues. The intracellular formation of VLDL in hepatocytes will be discussed in detail later. After VLDL enters the circulation, the particle is further enriched with apoE and apoCs[10]. Similar to chylomicrons, the TG content of VLDL can be lipolyzed into FA by LPL and used as energy source. VLDL-TG thus predominantly functions as a source of FA under fasting conditions. The processing of VLDL by LPL results in the formation of IDL (or VLDL-remnant) which can be further processed to become cholesterol-rich LDL[10]. The LDLr can bind and internalize both apoE and apoB containing particles[10]. VLDL remnants are predominantly cleared by the liver LDLr via apoE, whereas LDL is depleted from most apolipoproteins except for apoB and is cleared by the liver and peripheral LDLr[10]. High levels of apoB containing lipoproteins (chylomicrons, VLDL, IDL, and LDL) can lead to accumulation of lipids in the vascular wall and the development of atherosclerosis[13, 14].

Reverse cholesterol transport

HDL is responsible for the removal of excess cholesterol from peripheral tissues. In the liver and intestine, nascent HDL is formed from apoAI and phospholipids. The biosynthesis of HDL is dependent on the hepatic or intestinal ATP-binding cassette transporter A1 (ABCA1)[15]. In the circulation, HDL is enriched with phospholipids from chylomicrons and VLDL via phospholipid transfer protein (PLTP)[16], and cholesterol from the periphery via ABCA1, ABCGI and probably also scavenger receptor BI (SR-BI)[15, 17]. Cholesterol is subsequently esterified by lecithin:cholesterol acyltransferase (LCAT) into CE that are stored in the core of HDL[17]. The HDL particle expands due to cholesterol accumulation and matures into spherical HDL, which can acquire apolipoproteins including apoAII, apoAIV, apoAV, apoCI, apoCII, apoCIII and apoE[10, 18]. HDL-derived cholesterol can be taken up by the liver via SR-BI[19, 20, 21]. In the liver excess of cholesterol is secreted in the bile thereby maintaining cholesterol homeostasis[22].

The lipids in HDL can be exchanged with other lipoproteins through the interaction with PLTP to exchange phospholipids[16] and cholesteryl ester transfer protein (CETP)[23]. CETP is a glycoprotein that is mainly expressed

by liver, spleen, macrophages and adipose tissue[24]. CETP is predominantly associated with HDL in the circulation and mediates the exchange of CE and TG between apoB containing lipoproteins and HDL[17]. This results in a net flux of TG from (V)LDL to HDL in exchange for CE. The majority of CE from HDL is transferred to apoB containing lipoproteins thereby creating a more atherogenic profile with low levels of HDL-C and high levels of LDL-C. Furthermore, cholesterol transported to LDL by CETP can be taken up by the liver via the LDLr pathway thereby facilitating an alternative route for reverse cholesterol transport mediated by SR-BI.

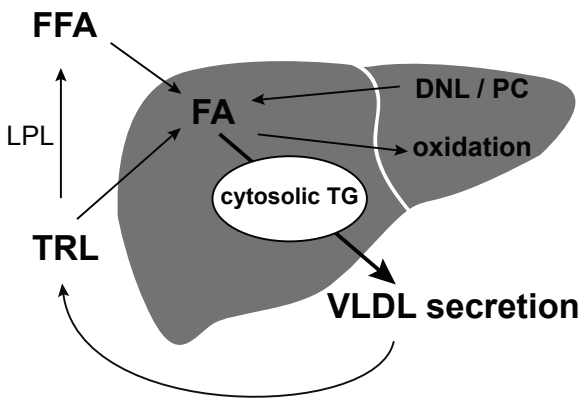
VLDL triglyceride and fatty acid metabolism

VLDL assembly

In the liver, TG is secreted in VLDL particles. The assembly of these particles starts in the endoplasmatic reticulum (ER) where newly synthesized apoB is cotranslationally lipidated by microsomal triglyceride transfer protein (MTP)[25, 26]. Each VLDL particle contains one apoB molecule. Since the transcription of apoB is relatively constant, regulation occurs at the posttranscriptional level. Whether apoB is targeted for degradation or lipidated is dependent on the availability of phospholipid and free cholesterol to form the surface monolayer, the availability of neutral lipids (TG and CE) to form the core and the presence of MTP which is necessary for the translocation, folding and lipidation of apoB[27]. When apoB is lipidated and targeted away from proteosomal degradation, a pre-VLDL particle is formed and released in the lumen of the ER where it can either be retained and degraded or further lipidated to form VLDL. This VLDL particle contains only a small amount of TG and is transported to the Golgi complex for secretion. In the Golgi, the VLDL particle can undergo a second step of lipidation in which the particle is loaded with bulk TG after which the mature TG-rich VLDL particle is secreted[25, 27].

The assembly of VLDL is highly dependent on the presence of TG in the hepatocyte. The origin of the FA of these TG is represented in figure 2. Most of the FA used for VLDL-TG secretion originates from the plasma. These FFA are released from the adipose tissue or are spill over from peripheral lipolysis of chylomicron-TG and VLDL-TG and taken up by the liver[28, 29]. Other sources of TG include previously accumulated cytosolic TG stores in the liver, receptor

mediated uptake of TG-rich lipoproteins (TLR), and TG esterification from *de novo* synthesized FA. *De novo* lipogenesis (DNL) in the liver is stimulated in the fed state[28]. FA are depleted from the liver by oxidation of FA (β-oxidation), which lowers the FA available for TG synthesis. A different source for the formation of TG is the phospholipid phosphatidylcholine (PC). Although phospholipids are mainly involved in structuring the VLDL particles, evidence accumulates that phospholipids themselves can also contribute to TG synthesis[30, 31, 32]. However the precise metabolic pathways involved in the conversion of phospholipids to TG are unknown.



F**igure 2. Schematic overview of sources of fatty acids and TG for VLDL synthesis.**
See text for explanation. DNL, *de novo* lipogenesis; (F)FA, (free) fatty acids; LPL, lipoprotein lipase; PC, phosphatidylcholine; TG, triglycerides; TRL, triglyceride-rich lipoprotein.

Lipoprotein lipase

Enzymes responsible for the hydrolysis of TG to FA and glycerol are collectively named lipases[33, 34]. Lipases hydrolyse the ester bonds of mono-, di-, and triglycerides, CE and phospholipids. This family of lipases consists of pancreatic lipase, present in the gut and necessary for the absorption of lipid by the intestine[7], adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), endothelial lipase (EL), hepatic lipase (HL) and LPL. LPL is expressed in most tissues, yet most abundantly in tissues that utilize FA for energy or storage (e.g. heart, skeletal muscle and adipose tissue)[33]. To become active, LPL is translocated to the luminal surface of endothelial cells lining the secreting tissues providing a platform for the interaction with TG-rich lipoproteins. This platform consists

of heparin sulfate proteoglycans (HSPGs)[12] and glycosylphosphatidylinositol-anchored high density lipoprotein binding protein 1 (GPIHBP1)[35]. Once TG-rich lipoproteins dock at the platform, LPL mediates the hydrolysis of TG and the released FA are for a large part taken up in the underlying tissue.

The regulation of LPL is tissue-specific and dependent on nutritional status. In the postprandial state, FA are primarily used for storage and therefore LPL activity is high in adipose tissue[36, 37]. During fasting, FA are primarily used as energy substrate and LPL activity is high in muscle[36, 37]. Several factors influence the activity of LPL, some of which are transported by the TG-rich lipoproteins themselves. ApoCII is an essential co-factor for LPL activity[38] and apoAV is a stimulator of LPL mediated lipolysis by guiding the lipoproteins to the lipolysis platform[39, 40]. However, apolipoproteins are also able to inhibit LPL. ApoCIII is a strong LPL inhibitor by affecting both the docking of TG-rich lipoproteins to the lipolytic site as well as directly inhibiting LPL itself[41]. ApoCI is also able to interact with LPL thereby inhibiting its activity[42]. Another group of inhibitors of LPL are the angiopoietin-like protein (Angptl) 3, that suppresses LPL activity and Angptl 4 that inhibits LPL by promoting the conversion of active LPL dimers into inactive LPL monomers[43]. These monomers can be released in the circulation thereby enhancing the binding and/or internalization of lipoproteins[44]. FA derived after lipolysis can be used for β-oxidation or stored in the form of TG by esterification.

Hormone sensitive lipase

In the fed state, adipose tissue is the major site of TG storage. There is a continuous cycle of lipolysis and (re-) esterification in the adipose tissue which is mediated by HSL and AGTL, which is termed the futile FA cycle[45, 46]. During intracellular lipolysis, generated glycerol diffuses to the circulation and FA can be released in the circulation or re-esterified to form TG again. The balance between lipolysis and re-esterification therefore determines the plasma levels of FA. During the fed state, the rate of lipolysis by HSL is inhibited by insulin[47] resulting in a net uptake of FA and accumulation of TG in the adipocytes. During fasting, HSL is stimulated by hormones such as glucagon[47] resulting in the release of FA into the circulation. These FA can be oxidized by other tissues, can be taken up by the liver for β-oxidation or can be metabolized into ketone

bodies that can serve as energy source for other tissues. FA from the circulation can also be re-esterified into TG and used as source for VLDL-TG production. This futile cycling of FA between liver and adipose tissue enables the body to adapt rapidly to changes in energy requirement.

Intracellular FA handling

FA are an important metabolic substrate but are extremely cytotoxic[3]. Therefore the uptake, transport and storage of FA is intensively regulated and plasma FA levels show relative little variation. After lipolysis, FA are taken up by the underlying tissue by passive diffusion across cell membrane or active transport which is facilitated by FA transporters such as FA translocase CD36[48] and plasma-membrane-associated FA-binding protein (FABPpm)[49]. In the cell, cytosolic FA binding protein (FABPc) and FA transport proteins (FATPs) target the FA to intracellular sites for conversion such as mitochondria for β-oxidation[50]. Diacylglycerol acyltransferases (DGATs) mediate the re-esterification of surplus FA so they can be stored as TG in intracellular lipid droplets. FA also act as important signalling molecules and modulate transcription factors to regulate the expression of genes involved in nutrient sensing and lipid metabolism. This is mediated by G-protein coupled receptors whereby GPR40 and GPR120 are activated by medium and long chain fatty acids[51], and GPR41 and GPR43 are activated by short chain fatty acids[52]. Activation of these FA receptors promotes the secretion of hormones involved in metabolism, such as insulin (GPR40[53], GPR120[54]), leptin (GPR41[55]) and GLP-1 (GPR120[56]), but can also influence our immune response (GPR43[57, 58], GPR120[54]).

Transcriptional regulation of TG and FA metabolism

Changes in the expression of genes involved in lipid metabolism are mediated by members of the nuclear receptor superfamily of ligand-dependent transcription factors. These nuclear receptors bind to target genes as heterodimers with retinoid X receptors (RXRs). In the presence of a ligand, these nuclear receptors recruit co-activator complexes resulting in the activation of transcription of the target gene[59]. In the absence of ligand, a co-repressor complex is recruited thereby preventing transcription. Binding of the ligand to the nuclear receptor replaces the co-repressor complex for the co-activator complex thereby

switching from repression to gene activation[59]. A third mechanism through which nuclear receptors act is by transrepression, involving the indirect binding of ligand-bound nuclear receptors to proteins instead of the target gene[59, 60] thereby enabling to control complex gene expression programs. There are several mechanisms through which transrepression can occur including competition and cross-coupling[61]. With competition the activated nuclear receptor competes for the binding of the co-activator complex in situations in which specific co-activators are limited. As a result, less co-activators are available for other transcription factors thereby inhibiting their gene activation. Cross-coupling on the other hand, involves the formation of a complex of the activated nuclear receptor with other activated transcription factors resulting in direct inhibition of each others transcriptional activity.

PPARs

Peroxisome proliferators activated receptors (PPARs) play a major role in lipid metabolism. This group of transcription factors consists of three different members that are activated by FA and eicosanoids. PPARα is highly expressed in metabolic active tissue such as liver, heart and muscle[62]. PPARα activation upregulates the expression of genes involved in TG hydrolysis (e.g. apoCIII[63] and LPL[64]) and in FA uptake (e.g. CD36)[65, 66] thereby lowering plasma TG levels. In the liver, PPARα also increases the expression of genes involved in FA import into mitochondria (e.g. CPT1)[67] and β-oxidation (e.g. acyl-CoA synthetase)[62], thereby reducing the intracellular FA concentrations. PPARγ expression is highest in adipose tissue where it affects the expression of genes involved in adipocyte differentiation[68, 69]. Furthermore, PPARγ has a major role in postprandial lipid metabolism. During the postprandial phase, PPARγ expression is highest[70] and upregulates the expression of genes involved in FA uptake and trapping[64, 66], resulting in the storage of lipids in adipocytes[71]. PPARδ is ubiquitously expressed[72] and enhances FA transport and oxidation. This results in depletion of triglyceride stores in tissues such as fat and muscle[73, 74]. Overall, PPARs act as lipid sensors and are able to regulate lipid homeostasis in multiple organs dependent on nutritional and/or energy status.

SREBP

Sterol regulatory element binding proteins (SREBPs) are also major regulators of lipid metabolism and are activated when cells become depleted of cholesterol[75]. There are three isoforms, SREBP1a and SREBP1c that share the same gene and SREBP2. SREBP1c is an important downstream target for both the liver X receptor (LXR)[76] and farnesoid X receptor (FXR)[77] and is the major regulator of hepatic FA and TG metabolism[78]. When carbohydrates and saturated FA are abundant, SREBP1c expression is activated and TG storage is increased[78]. While during fasting, SREBP1c is inhibited and FA oxidation is increased[78].

Xenobiotic receptors

Activation of the xenobiotic receptors by environmental chemicals and drugs induces the expression of proteins important for the metabolism, deactivation and transport of these chemicals. However, pathways used for xenobiotic metabolism overlap with pathways involved in lipid metabolism. Constitutive androstane receptor (CAR) and pregnane X receptor (PXR) both belong to the nuclear receptor family and decrease the expression of genes involved in β-oxidation[79, 80, 81]. Furthermore, PXR increases the uptake of FA in the liver of mice by increasing the expression of CD36 and increases the hepatic expression of lipogenic genes[82]. Overall activation of xenobiotic receptors can lead to changes in hepatic and plasma lipid profiles[83, 84, 85] and PXR or CAR activating drugs can even lead to lipid accumulation in the liver of patients[86, 87].

PGC-1

One of the co-regulators of nuclear receptors involved in the control of energy metabolism is the PPARγ coactivator-1 (PGC-1) family. Several targets of PGC-1 are PPARs[73, 88], PXR[89], CAR[90] and also non-nuclear receptors such as SREBP-1[91] and forkhead box O1 (FOXO1)[92]. In the liver, PGC-1 signalling is increased during fasting and activates gene expression involved in FA oxidation by co-activating PPARα[92, 93]. This increase in FA oxidation is required to produce substrates necessary for the production of glucose and is mediated through PGC-1α and PGC-1β[91, 92, 93]. PGC-1β also activates the expression of genes involved in lipogenesis and VLDL production by co-activating SREBP1c[91].

The association between lipid and glucose metabolism

In addition to lipids, carbohydrates are an important source of energy in our diet. Complex carbohydrates such as starch are digested into glucose prior to absorption in the gut. After a meal, blood glucose levels rise, which results in the release of insulin into the circulation by the pancreas. Insulin is a central anabolic hormone that regulates both glucose and lipid metabolism[94]. The main action of insulin is to decrease excessive glucose by stimulating metabolism and storage. The liver is a central player in glucose homeostasis, since it can store glucose in the form of glycogen for later use[95]. However, due to limited glycogen storage insulin promotes *de novo* lipogenesis from excessive glucose to produce FA.

Under fasting conditions, blood glucose levels drop and since the brain is highly dependent on glucose, glucose levels need to be maintained. In response to glucose lowering, insulin is no longer produced by the pancreas to prevent further storage of glucose. In addition, glucose oxidation is lowered and FA oxidation increased in tissue, thereby preventing further usage of glucose. The drop in glucose levels stimulates the production of glucagon by the pancreas. Under the influence of glucagon, glucose can rapidly be mobilized from hepatic glycogen stores (glycogenolysis) and released into the circulation to provide tissues with glucose that can not function without it, such as the brain. When these glycogen stores become depleted, the liver starts producing glucose from substrates such as amino acids and glycerol (gluconeogenesis) to maintain blood glucose levels[96]. During prolonged fasting, glucose is no longer readily available and ketone bodies are used as energy source instead. Ketone bodies are produced in the liver from fatty acids, a process known as ketogenesis, and provide an alternative energy source for tissues, such as the brain, to maintain its function.

Pathology of lipid and glucose metabolism in the metabolic syndrome

Since lipid and glucose metabolism are tightly linked, disturbances in one metabolic pathway in general also involves the other. Excessive calorie intake and a diet rich in saturated fat predisposes to obesity and concomitantly dyslipidemia, which is characterized by high levels of TG (hypertriglyceridemia), high levels of small, dense LDL-C and low levels of

HDL-C. This is also known as the metabolic syndrome, which refers to a cluster of correlated disorders including obesity, dyslipidemia, insulin resistance and hypertension[97]. The metabolic syndrome but also all the various components themselves are associated with an increased risk to develop DM2 and CVD[98, 99, 100]. The metabolic syndrome comprises storage of excess TG in non-adipose, resulting in unresponsiveness of this tissue to insulin, also known as insulin resistance[101, 102, 103]. Insulin resistance of the liver results in impaired repression of glucose production in response to insulin, resulting in high blood glucose. To compensate for insulin resistance, the pancreas starts to produce more insulin to maintain normal glucose levels. When the demand on the pancreas to produce insulin exceeds its capacity, the pancreas is damaged, leading to DM2.

A combination of increased storage of TG in the liver and insulin resistance is associated with increased secretion of VLDL-TG[104, 105]. In addition, insulin resistance is also associated with increased lipolysis of TG in adipose tissue by HSL resulting in more FA flux from adipose tissue to the liver, thereby increasing the liver TG stores. The increased production of VLDL-TG by the liver can result in hypertriglyceridemia, thereby providing more apoB lipoprotein acceptors for the transfer of cholesterol by CETP. As a result HDL becomes depleted of cholesterol and LDL-C levels increase[106].

A high level of LDL-C is the major cause of atherosclerosis. Cholesterol rich LDL particles can invade the arterial wall where they are oxidized and cause local damage[13, 14]. Monocytes are attracted to the site of damage and differentiate into macrophages that can incorporate the LDL particles thereby becoming cholesterol rich foam cells[13, 14]. The accumulation of these cholesterol rich foam cells results in the formation of plaques. Rupture of these plaques can lead to an infarct, damaging surrounding tissue[13, 14].

Models to study lipoprotein metabolism

Dyslipidemia in humans is a multifactorial disease with both genetic and environmental origins. Due to the complexity of many dyslipidemias, animal models have been developed to investigate specific components of dyslipidemia to gain more insight in the mechanisms involved. Mice are a commonly used model since they are easy to breed, genetically homogeneous and their environment and diet is easily controlled. Moreover, mice can be

genetically manipulated by both changing the expression of endogenous genes and by introducing novel genes. Unfortunately, lipid metabolism in mice is somewhat different compared to humans. Mice are very efficient in clearing apoE-containing lipoproteins from circulation, which results in low levels of apoB containing lipoproteins such as VLDL and LDL. In addition, mice lack the enzyme CETP and have high levels of HDL compared to humans.

Several mouse models have been developed which are more similar to human lipoprotein metabolism such as the LDL receptor-deficient ($LDLr^{-/-}$) and apolipoprotein E-deficient ($ApoE^{-/-}$) (reviewed in[107]). The mouse model used in this thesis to study TG and FA metabolism is the transgenic ApoE*3-Leiden (E3L) mouse. E3L mice carry the human apoCI gene and a variant of the human ApoE*3 gene which causes a genetic form of hyperlipidemia in humans[108, 109]. Expression of these transgenes in E3L mice is associated with decreased LPL activity, a disturbed interaction of lipoproteins with the LDLr and LRP and impaired hepatic clearance of apoE containing lipoproteins. Together this is associated with a more human-like lipoprotein metabolism that is characterized by elevated levels of VLDL and LDL.

On a standard chow diet, E3L mice have moderately increased levels of plasma TG and cholesterol. However, E3L mice are highly responsive to diets rich in fat and cholesterol, resulting in strong increases in plasma TG and cholesterol levels[110]. The E3L mice have been used to study (V)LDL metabolism in response to various hypolipidemic drugs. In contrast to the often used $LDLr^{-/-}$ and $ApoE^{-/-}$ hyperlipidemic mouse models, E3L mice have been shown to respond in a human-like manner to a large number of drugs that modify LDL-C. These drugs include statins[111, 112, 113], fibrates[114, 115] and cholesterol uptake inhibitors[115]. However, mice naturally lack CETP, important for both (V)LDL and HDL cholesterol metabolism. Therefore E3L mice have recently been crossed with mice expressing human CETP under control of its own promoter. The resulting E3L.CETP transgenic mice resemble human lipoprotein metabolism even more closely compared to E3L mice by the redistribution of cholesterol from HDL to (V)LDL[116]. As a result, these E3L.CETP mice respond to both LDL and HDL modifiers[85, 114, 117, 118, 119] and are a valuable model to study the effects of pharmaceuticals on lipoprotein metabolism.

Outline

The research in this thesis focuses on the regulation of TG and FA metabolism to get a better understanding of TG and FA metabolism in health and disease. The effect of CETP on TG metabolism is studied by using the E3L mouse model in **Chapter 2**. Previous studies have shown that expression of CETP in E3L mice results in a shift of cholesterol from HDL to apoB containing lipoproteins. Since CETP simultaneously exchanges CE with TG, we set out to evaluate whether the impact of CETP on TG metabolism is similar to the impact of CETP on cholesterol metabolism.

The mechanisms underlying the TG-lowering effect of the PPARα agonist fenofibrate are studied using E3L.CETP mice in **Chapter 3**. This TG-lowering effect of fenofibrate has been attributed to both increased TG clearance and decreased VLDL-TG production. However, since data on the effect of fenofibrate on VLDL production are controversial, we aimed to investigate the mechanism underlying the TG-lowering effect of fenofibrate.

The effects of the antibiotic rifampicine on TG metabolism are discussed in **Chapter 4**. This drug is a potent PXR activator that induces hepatic steatosis in both humans and rodents.

Chapter 5 describes the effect of perfluoroakyl sulfonates (PFAS) on lipid metabolism. PFAS are very useful for water and oil repellence, but are therefore also extremely resistant to degradation. As a consequence PFAS accumulate in the environment and can be found in the blood of both wildlife as well as humans. PFAS are considered to act as PPARα agonist thereby affecting lipid metabolism. Our aim was to investigate the effects of PFAS on both TG and cholesterol metabolism in E3L.CETP mice.

Hepatic steatosis affects both lipid and glucose metabolism and is associated with insulin resistance. In mice, both prolonged fasting and feeding a high fat diet induces hepatic steatosis, however only after a high fat diet, hepatic steatosis is associated with insulin resistance. **Chapter 6** describes the differences in the hepatic gene expression profile of mice fasted for 16 hours compared to mice fed a high fat diet for 2 weeks.

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