Hepatic steatosis : metabolic consequences

Boer, A.M. den

Chapter 1

General Introduction

Hepatic steatosis refers to the condition of accumulation of triglycerides (TG) in hepatocytes. From a quantitative perspective this storage capacity of the hepatocytes is much less important than the accumulation of TG in adipocytes. TG accumulation in the liver was thought to be an inert histological epiphenomenon. However, nowadays we know that hepatic steatosis is associated with several metabolic changes in lipid and glucose metabolism not only in the liver, but also throughout the body. In this introduction the emphasis is on TG metabolism since TG are the most important lipids that are involved in hepatic steatosis. The regulation of TG metabolism is described with special focus on the causes and consequences of hepatic steatosis.

Whole body triglyceride and fatty acid metabolism

Dietary triglycerides are absorbed in the intestines and packed into chylomicrons. Chylomicrons are very large particles that contain mainly TG, but also consist of phospholipids, cholesterol and proteins.[1] Upon secretion from the intestines into the lymph and subsequently into the bloodstream, these large TG-containing particles acquire apolipoprotein (apo-)B, E and apoCI, -II and III on their surface. The liver also produces TG-rich lipoproteins, i.e. very-low density lipoproteins (VLDL-TG). These VLDL-TG particles also contain cholesterylesters in the hydrophobic core.[1] The surface monolayer consists of cholesterol, phospholipids and protein. In addition to a single apoB molecule per VLDL-TG particle, the shell of the particles is enriched in apoE and apoCI, -II and -III upon secretion into the circulation.

In the fed state a mixture of VLDL-TG (from the liver) and chylomicrons (from the intestines) enters the circulation, where these TG particles are subject to lipolysis by endothelium-bound lipoprotein lipase (LPL), as shown in Figure 1.[2,3] LPL is synthesized in, and secreted by, parenchymal cells throughout the body. It is most abundant in cardiac and skeletal muscle and adipose tissue. Several apolipoproteins influence the lipolytic conversion by LPL. ApoCII is an activating co-factor for LPL[4,5], whereas apoCI and apoCIII inhibit lipolysis.[6,7] In addition, high amounts of apoE can inhibit LPL-mediated lipolysis.[8,9] The process of local lipolysis by LPL generates fatty acids (FA) that can enter the adipose tissue, muscle or the heart, either for energy provision via β-oxidation or for TG storage, depending on the oxidative requirements of the respective tissues. LPL expression is regulated by tissue-specific mechanisms, that also depend on hormonal and nutritional status.[3,10-12] LPL activity decreases

during fasting and increases after a meal containing fat.[13-15] Postprandially, LPL is abundantly expressed on adipose tissue, whereas during fasting the expression on skeletal muscle increases.[10-12]

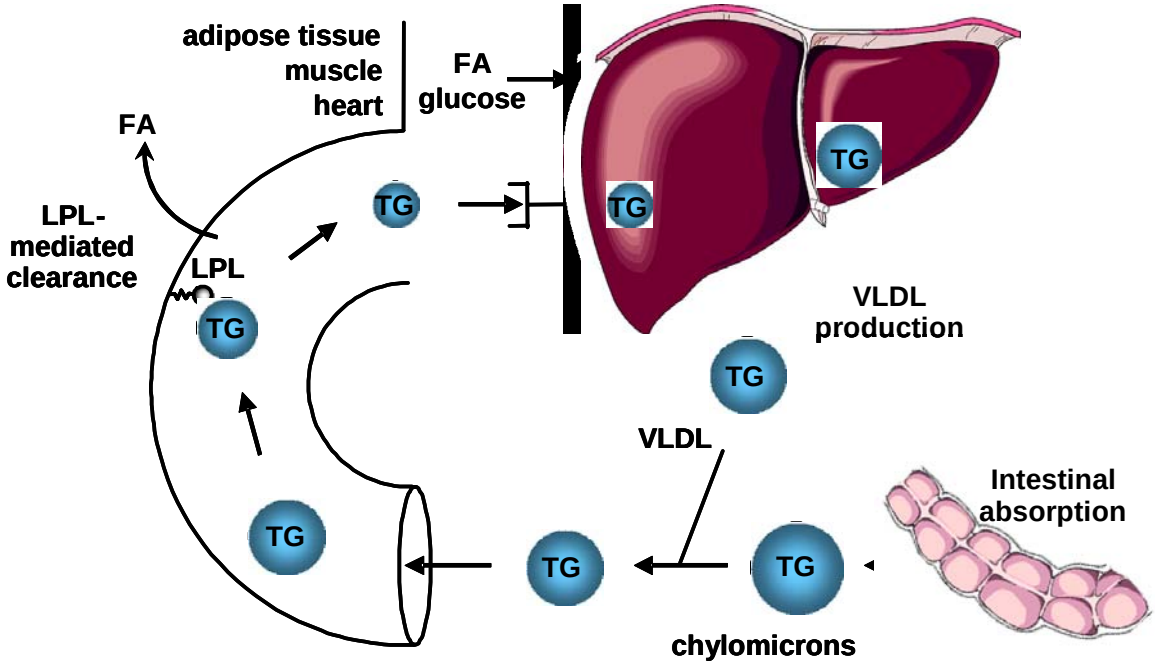

Figure 1. Schematic representation of whole-body TG metabolism. Chylomicrons from the intestines and VLDL-TG from the liver enter the circulation. In the capillaries these particles are lipolyzed by lipoprotein lipase (LPL). The generated FA enter the cardiac and skeletal muscle and the adipose tissue where they can be stored as TG or used for energy provision. After several lipolysis steps the remnant particles are taken up by the liver and further processed.

Hydrolysis of VLDL-TG results in the formation of intermediate density lipoproteins (IDL) and subsequently low density lipoproteins (LDL). In addition to LPL, hepatic lipase (HL) is responsible for further hydrolysis of the particles.[16] After several lipolysis steps, these remnant particles are recognized by the liver by their apoB and apoE, taken up by specific lipoprotein receptors such as LDL-receptor (LDLr) and LDLr-related protein (LRP) and further processed.

Adipose tissue is an important regulator of triglyceride metabolism. It acquires FA from the circulation, either from the free FA pool or from FA derived from plasma TG, through the activity of LPL. Moreover, TG contained within adipose tissue are continuously hydrolyzed by hormone sensitive lipase (HSL) and other lipases[17], which results in the release of FA into the plasma. These FA can subsequently either

be oxidized in other tissues, or be taken up by the liver. Within the liver these FA have two fates: oxidation or re-esterification into TG and subsequent release into the plasma in the form of VLDL-TG. Consequently, there is a futile cycle of FA between adipose tissue and the liver, enabling the body to adapt rapidly to changes in energy requirements. In pathophysiological conditions, in which there are disturbances in this cycle, accumulation of TG may occur in the liver. Since there is a huge fat mass in relation to the very limited maximum storage capacity of TG in the liver, it is likely that only minor changes in fatty acid cycling may result in liver steatosis. This is illustrated by a rapid increase of liver TG after skipping just a few meals during short term starvation.

Hepatic triglyceride and fatty acid metabolism

The liver plays a central role in lipoprotein metabolism. It produces, secretes and takes up lipoproteins. In FA metabolism the liver also plays an important role: it is involved in FA uptake from plasma, FA oxidation, *de novo* FA synthesis, and VLDL-TG secretion. Moreover, FA and their derivatives have major effects on expression levels of many genes, because these FA serve as ligands for several transcription factors which are crucial in the regulation of glucose and fat metabolism.

Hepatic TG metabolism

Hepatic VLDL-TG production (HVP) is mainly substrate-driven, but it is also determined by the hormonal and nutritional status of the individual. VLDL-TG assembly and secretion is a two-step process as described by Alexander et al. in 1976.[18] The first step is the association of one apoB with the core lipids. Microsomal TG transfer protein (MTTP) forms an important step in VLDL-TG-assembly, since it catalyzes the transfer of lipids towards the apoB molecule. This particle fuses with a lipid droplet and generates a mature VLDL-TG particle. The flux of FA to the liver and the amount of TG in the liver are factors influencing HVP. However, this relationship is not always straightforward. The amount of TG accumulated in the liver is not a direct determinant of the production of VLDL-TG. In rats it has been shown that acute stimulation of *de novo* lipogenesis leads to steatosis without affecting VLDL-TG production.[19] Moreover, increased FA flux to the liver does not always lead to increased VLDL-TG production. For instance, CD36 knockout mice have a 60% increase in hepatic TG content[20], but no change in hepatic VLDL-TG production.[21]

Obviously, other factors than merely the availability of FA control hepatic VLDL-TG production. Hormonal effects on HVP will be discussed later on in this introduction.

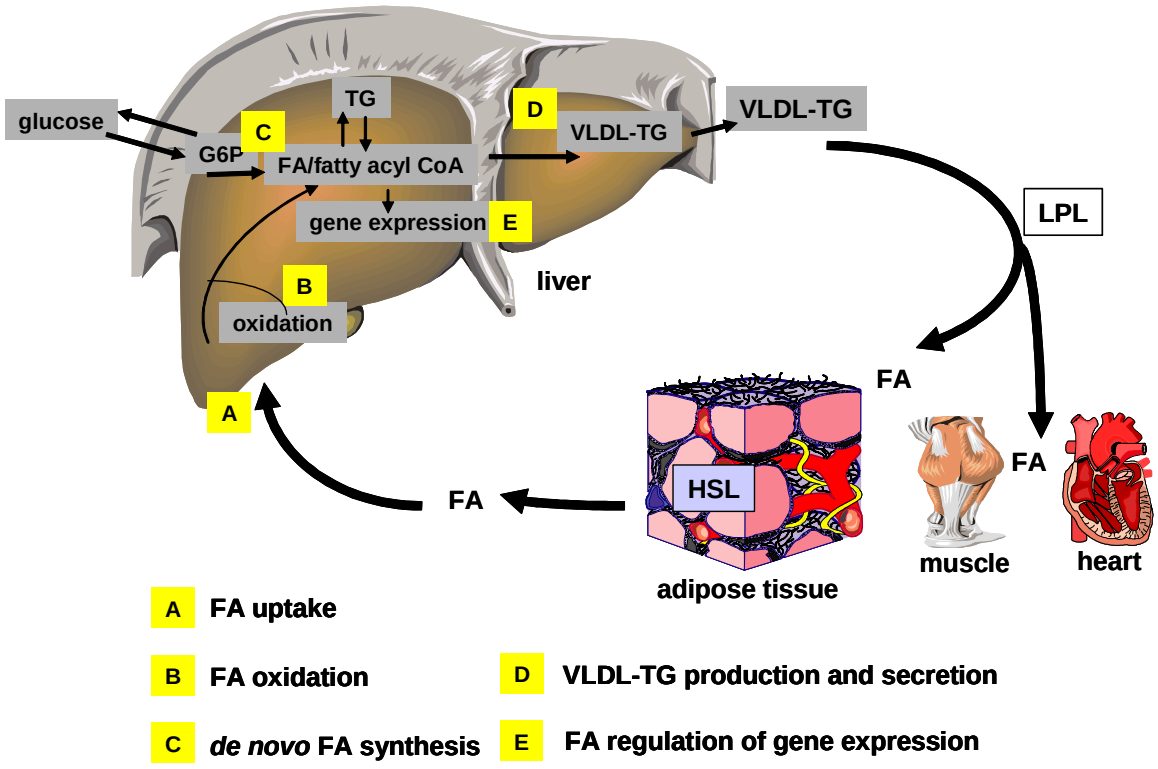

Figure 2. Major pathways of hepatic fatty acid/triglyceride metabolism in the liver. The liver plays a central role in lipid metabolism through **A.** Uptake of fatty acids, **B.** Fatty acid oxidation, **C.** *De novo* fatty acid synthesis, **D.** Assembly and secretion of VLDL-TG, **E.** Effects of fatty acids on gene expression. FA = fatty acids, HSL = hormone sensitive lipase, LPL = lipoprotein lipase, G6P = glucose-6-phosphate.

Hepatic FA metabolism

The liver is important in FA metabolism, because it takes up FA from the circulation (Figure 2). Most medium- and long-chain FA that enter the liver are oxidized in the mitochondrial β-oxidation system. Very-long-chain FA are mainly oxidized in the peroxisome.[22-24] During fasting FA that enter the liver can be metabolized via acetyl coenzyme A (acetyl CoA) to form ketone bodies that can serve as fuel for other tissues such as the brain.[25,26] When the flux of FA towards the liver exceeds β-oxidation capacity, this can lead to accumulation of TG in the liver. FA in the liver are continuously being re-esterified into TG. TG from this pool can be used for hepatic VLDL-TG synthesis and secretion. Actually, most of the plasma VLDL-TG pool is derived from plasma FA, re-esterified by the liver and secreted into the plasma,

whereas only a very small fraction of plasma VLDL-TG is derived from *de novo* fatty acid synthesis in the liver.[27]

Hepatic glucose metabolism

Glucose is the most important energy source in the mammalian body. Especially the brain is depending primarily on readily available glucose. Therefore, it is very important for the body to control plasma glucose concentrations tightly. The plasma glucose level is determined by the balance between dietary uptake in the intestines, glucose uptake by peripheral tissues and the production of glucose by the liver. The liver plays a very important role in the glucose homeostasis by controlling the balance of appearance and disappearance of glucose.

Storage of glucose

Excess glucose is stored as glycogen (glycogenesis) which is a very efficient storage form of glucose.[28] In the liver hexokinase, also known as glucokinase, mediates the first step of hepatic glucose metabolism, which involves the conversion of glucose into glucose-6-phosphate (G6P). G6P is an important metabolic intermediate in the glucose metabolism.[29] In the second step glycogen is produced via uridine diphosphate (UDP-) glucose. In this step glycogen synthase is the rate-controlling enzyme. Not all glucose can be stored as glycogen since the liver's storage capacity is limited. The excess glucose is broken down to pyruvate and lactate so that it can be used in *de novo* lipogenesis to produce FA.

Production of glucose

During fasting the mammalian body depends on the liver and (to a much lesser extent) the kidneys for production of glucose.[30] In the early phase of fasting the liver produces glucose by hydrolysing glycogen via 3 steps (glycolysis). First, a single glucose-1-phosphate is cleaved from glycogen mediated by glycogen phosphatase and then a debranching enzyme converts glucose-1-phosphate to G6P. Finally, G6P is dephosphorylated to glucose and this process is under control of glucose-6-phosphatase (G6Pase).[31] The liver can also form glucose from alternative substrates such as amino acids, glycerol, pyruvate and lactate to maintain stable blood glucose levels (gluconeogenesis).[32] Phosphoenolpyruvate carboxykinase (PEPCK) controls the regulation of the latter process. To protect against complete breakdown of the

glycogen stores, the liver progressively increases gluconeogenesis during prolonged periods of fasting.[33]

Insulin action

Insulin is a key regulator in both glucose and lipid metabolism. It is the most important hormone controlling hepatic glucose and VLDL-TG output.[34] Insulin is a 5.8 kDa hormone secreted by the β-cells of the islets of Langerhans in the pancreas. Insulin is secreted in a biphasic manner in response to an increase in blood glucose level. There is an initial burst of insulin secretion that lasts about 5-15 minutes, resulting from secretion by the preformed insulin secretory granules. This initial burst is followed by a more gradual and sustained insulin secretion resulting from biosynthesis of new insulin molecules.

The binding of insulin to the insulin receptor leads to a phosphorylation cascade eventually resulting in the induction of several target genes.[34] In addition to glucose, amino acids, long-chain FA and several hormones can induce insulin secretion. The main target organs for insulin are the liver, skeletal muscle and adipose tissue. The overall net result of insulin action is fuel storage of both glucose and lipids. Insulin resistance refers to the condition where a specific tissue is (or several tissues are) less sensitive to the effects of insulin.

Insulin exerts direct effects on VLDL-TG production, although the mechanism behind this phenomenon remains unclear.[35] Insulin is thought to accelerate the degradation of apoB[36] and VLDL-TG secretion is decreased when apoB availability is decreased. Insulin indirectly inhibits HVP by inhibiting HSL. The consequence of decreased TG-hydrolysis by HSL is a decreased flux of FA towards the liver resulting in decreased substrate availability for hepatic VLDL-TG output.

Hepatic glucose output (HGO) is determined by the rate of hepatic glycogen breakdown, which is regulated by G6P and by the rate of hepatic gluconeogenesis which is regulated by PEPCK. [37-39] In the fed state insulin inhibits HGO via inhibition of these two key regulatory enzymes. Insulin also stimulates glucose uptake by peripheral tissues (PGU) such as the muscle and adipose tissue. In these tissues insulin stimulates translocation of the glucose transporter-4 (Glut-4) mediating uptake of glucose.[40]

Interactions of glucose and lipid metabolism

In the liver, glucose and lipid metabolism are closely linked. In the presence of decreased glucose availability, glucose oxidation decreases and the need for the oxidation of FA increases.

Substrate availability

In 1963 the glucose/FA cycle was postulated by Randle.[41] Based on experimental evidence, this cycle states that the availability of FA determines the rate of FA oxidation and that FA oxidation directly inhibits glucose oxidation. The exact mechanism behind this interaction has not been elucidated. Several mechanisms have been proposed to explain this link between FA and glucose oxidation including the accumulation of intermediates in the FA and glucose metabolism.[42]

Some studies investigated the effects of increasing plasma FA by infusion but observed no effects on the intermediates such as citrate or G6P levels.[43-45] On the other hand the infusion of lipids or FA can induce insulin resistance leading to decreased uptake of glucose.[46] However, this does not automatically include decreased glucose oxidation. When plasma FA levels were increased during hyperinsulinemic hyperglycemic clamp conditions, no effects on glucose oxidation were observed.[47] Therefore, it was proposed that glucose availability may be the most important determinant for substrate utilisation.[48]

Transcription factors

FA derivatives can exert significant effects on transcription factors.[49] FA activate PPARα by direct binding, leading to the induction of hepatic FA oxidation.[50] FA can inhibit hepatic FA synthesis by indirectly suppressing sterol responsive element binding protein-1c (SREBP-1c), which can be induced by insulin. Fatty acid control of this transcription factor is not completely clear yet. On the other hand, glucose can activate carbohydrate responsive element binding protein (ChREBP).[51,52] Most lipogenic enzymes have response elements for binding ChREBP (ChRE) and SREBP (SRE). These two factors work synergistically to induce transcription of the lipogenic enzyme genes in the presence of glucose and insulin. Glucagon and FA can inhibit the activation of the ChRE and SRE. In this way the control of expression of lipogenic enzyme genes is regulated in an integrated manner by multiple nutrient and hormonal signals.

Taken together, FA and glucose control hepatic lipid composition and the type and quantity of lipids available for hepatic VLDL-TG production. Because the liver plays a central role in lipid metabolism these transcription factors can affect whole-body lipid composition. Ultimately, increased plasma levels of FA and glucose contribute to the onset and progression of chronic diseases such as atherosclerosis, diabetes and obesity.[53] It may well be that the interactions between glucose and FA metabolism may be dependent on the circumstances and on tissue specific mechanisms. Nevertheless, all evidence points towards important interactions between the glucose and FA metabolism.

Hepatic steatosis

TG from the diet are mainly stored in adipose tissue. These TG form the most important energy storage in mammals. In humans about 10-30% of the body weight is adipose tissue. It provides the body with a virtually limitless capacity to store TG, which is reflected by extreme forms of obesity. TG storage is evolutionary very important to allow survival during periods when food is scarce. In addition to adipocytes, the liver and skeletal muscle can accumulate TG, although to a much lesser extent. Hepatic steatosis, or fatty liver, is defined as a histopathological condition marked by increased accumulation of lipids within the hepatocytes. Several forms of hepatic steatosis can be distinguished, depending on the underlying condition and progression of the disease. Although it has been known for a long time that excessive alcohol use is associated with hepatic steatosis we focus on non-alcoholic steatosis in this thesis.

Facts and figures

In the body there is a continuous cycling and redistribution of non-oxidized FA between different organs especially in the post-absorptive state, with a central role for the interaction between the liver and the adipose tissue. When the input of FA into the liver exceeds the FA oxidation and output of VLDL-TG, hepatic steatosis occurs. During fasting or high fat feeding, hepatic steatosis can be readily induced in healthy subjects. The flexibility of the liver in the accommodation of TG had already been demonstrated in dogs in 1970.[54] Hepatic steatosis is observed frequently even in normal-weight and moderately overweight subjects.[55] The prevalence of fatty liver in the general population is estimated to be 3% to 24%, with most estimates in the 6%

to 14% range.[56] In obese and diabetic subjects the prevalence of hepatic steatosis is estimated to be much higher.[57] Patients eligible for bariatric surgery have a body mass index of ≥ 40 kg/m^2 or of ≥ 35 kg/m^2 with significant co-morbidities such as type 2 diabetes. In this population the prevalence of fatty liver is estimated to range even from 84% to 96%.[56] Nowadays, we know that in about half of the subjects hepatic steatosis can progress to fibrosis, 15% progress to cirrhosis and 3% eventually experience liver failure or need a liver transplant.[58] Apart from progression to these more severe stages of liver disease, hepatic steatosis is associated with a number of metabolic disturbances in glucose and lipid metabolism in the liver and even throughout the body. However, it remains unclear whether these disturbances are a cause and/or a consequence of the TG accumulation.

Causes of hepatic steatosis

The accumulation of TG can be caused by an increased mobilization and increased availability of FA in the circulation.[59] HSL activity in adipose tissue is regulated among others by insulin. In insulin resistant states insulin no longer (or to a lesser extent) inhibits HSL, causing too many FA to be released into the circulation.[34,60] The liver functions as a buffer and takes up the excess FA. Epinephrine and norepinephrine stimulate the mobilization of FA from adipose tissue by stimulating HSL.[61] A high fat diet or long term fasting can also cause an increased flux of FA to the liver. The CD36 knockout mouse is a mouse model that lacks the FA transporter CD36 in muscle and adipose tissue, causing an increased flux of FA to the liver. These mice show hepatic steatosis and have severely insulin resistant livers.[20]

Increased *de novo* lipogenesis in the liver can cause TG accumulation in hepatocytes.[19,59,62] In this process FA are produced from glucose. Rate-limiting enzymes in *de novo* lipogenesis are acetyl-coenzymeA carboxylase (ACC) and fatty acid synthase (FAS). These enzymes are stimulated under fed conditions by insulin and high carbohydrate diets. Glucagon inhibits endogenous FA synthesis. Cortisol inhibits FAS and endogenous FA synthesis in the liver while it stimulates TG lipolysis in the circulation by stimulating LPL activity.

Increased esterification of FA can also lead to TG accumulation.[59] TG in the hepatocytes are not an inert storage but are continuously recycled.[63] Intracellular TG are lipolyzed in larger quantities than necessary to form VLDL-TG. The FA that are

not build into VLDL are re-esterified into TG and are transported back into the cytoplasmatic pool. When this equilibrium is disturbed, TG accumulation can occur.
Decreased secretion of VLDL-TG by the liver can cause accumulation of TG. VLDL-TG production is regulated by several factors as has been discussed previously. An important factor regulating VLDL-TG production is the size of the intracellular TG pool, but limited synthesis or availability of any of the important components of VLDL-TG can inhibit the production of the particle. ApoE stimulates the secretion of VLDL-TG.[64]
Finally, decreased mitochondrial β-oxidation can be the cause of hepatic steatosis.[65] Studies in children with inborn deficiencies in one or more enzymes of the FA oxidation pathway have shown that, when there is a need for increased β-oxidation during short term fasting (for example during infection), this often ends fatal with an enormous accumulation of TG in the liver.[66] Insulin inhibits and glucagon stimulates mitochondrial β-oxidation in the liver.
Taken together, the accumulation of TG within hepatocytes is caused by a disturbed equilibrium between liver TG synthesis and secretion.[67] An increased flux of FA to the liver from adipose tissue, dietary intake or endogenous synthesis can lead to accumulation of TG in the hepatocytes when mitochondrial β-oxidation and VLDL-TG secretion and production are not capable of processing all incoming FA.

Metabolic consequences of hepatic steatosis

Hepatic steatosis is not only a consequence of metabolic disturbances, but steatosis *per se* can also have profound effects on lipid and glucose metabolism and cardiovascular risk factors. Accumulation of TG in the liver is strongly associated with hepatic insulin resistance.[68] This is associated with cardiovascular risk factors such as hyperglycemia, hypertriglyceridemia, and elevated levels of alanine transferase (ALT), fibrinogen, C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1) and factor VII (Figure 3).[68]

Effects on lipid metabolism

In insulin resistant states the liver becomes less sensitive to the inhibitory effect of insulin on hepatic VLDL-TG production. This contributes to dyslipidemia in insulin resistant states. The inability of insulin to accelerate the degradation of apoB results in the overproduction of VLDL-TG particles. This contributes to the frequently

observed diabetic hypertriglyceridemia. Insulin also has peripheral effects on the lipid metabolism, since it normally regulates the expression of LPL, resulting in a net storage of lipids into the adipose tissue. In insulin resistant states VLDL-TG particles remain longer in the circulation because insulin does not (or to a lesser extent) induce LPL-expression. This allows more transfer of TG to LDL and HDL particles by cholesteryl ester transfer protein (CETP).[69] When CE-depleted TG-rich LDL particles are hydrolyzed by LPL and HL, this leaves small dense LDL particles which are highly atherogenic. Eventually the frequently observed diabetic dyslipidemia is established that poses an increased risk for cardiovascular disease.

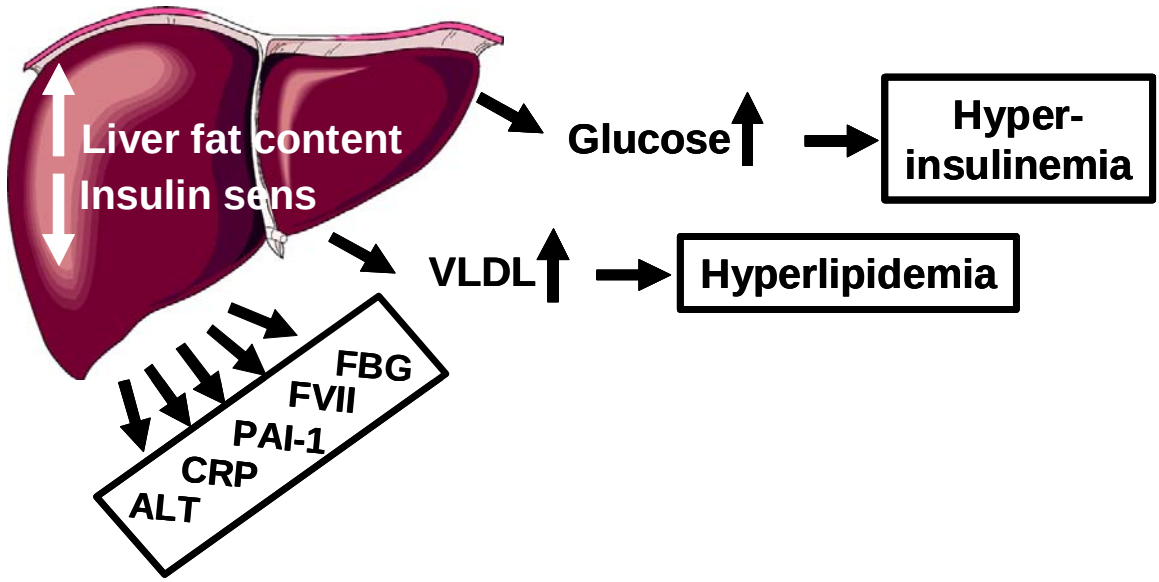

Figure 3. The fatty liver overproduces cardiovascular risk factors. Hepatic steatosis is strongly associated with insulin resistance. The insulin resistant liver overproduces glucose, VLDL-TG and other factors known to associate with enhanced cardiovascular risk such as alanine transferase (ALT), fibrinogen (FBG), C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1) and factor VII (FVII).

Effects on glucose metabolism

Decreased insulin sensitivity associated with increased hepatic lipid content has major effects on the glucose metabolism. The suppressive effect of insulin on G6Pase and PEPCK expression levels is decreased. This will lead to more glycogen breakdown and more gluconeogenesis and consequently increased hepatic glucose output. Postprandially insulin normally decreases the output of glucose and increases uptake of glucose by peripheral tissues. Consequently, plasma glucose levels are

well controlled. In subjects with fatty liver a higher output of glucose may exist.[68] Because insulin action is impaired postprandially (decreased suppression of liver output) the glucose output is less suppressed by insulin. Peripheral tissues still take up glucose, however the increased (relatively) steady glucose levels cause diabetic adverse effects. The pancreas compensates by increased insulin secretion (hyperinsulinemia). In time β-cell failure will occur and the body can no longer compensate for insulin resistance with extra insulin secretion. This results in hyperglycemia despite hyperinsulinemia and this state is referred to as type 2 diabetes mellitus.

Outline of this thesis

The studies described in this thesis focus on the metabolic consequences of hepatic steatosis on lipid and glucose metabolism. Many interactions between the glucose and lipid metabolism exist. Research usually tends to focus on glucose metabolism with regard to insulin resistance and type 2 diabetes mellitus. However, concomitant disturbances in lipid metabolism may be of great importance for the major clinical endpoints such as cardiovascular disease. In this thesis we investigated the integrated role of the glucose and lipid metabolism from the perspective of the liver.

Hepatic steatosis is frequently observed and is associated with a number of cardiovascular risk factors.[70-73] From observations in humans it remains unclear to what extent hepatic steatosis is a cause rather than a consequence of the metabolic syndrome. In this thesis several mouse models of steatosis with targeted disruptions of the fatty acid and TG metabolism are used to study these causes and consequences of fatty liver. We reviewed reported studies in rodent models of hepatic steatosis in Chapter 2.

Since in humans the liver is not readily accessible and study protocols can be limiting, mouse models are often used to investigate mechanisms of insulin resistance. The C57/Black 6 mouse is a wild type mouse model that is sensitive to diet-induced hyperlipidemia, obesity and insulin resistance.[74,75] In this model we compared the inhibitory effects of insulin on hepatic glucose and VLDL-TG production in Chapter 3.

In Chapter 4 the CD36-deficient mouse (*cd36* $^{-/-}$) is studied. This mouse completely lacks CD36 or Fatty Acid Transporter (FAT) in adipose tissue and muscle and cannot take up FA in these peripheral tissues.[83] Consequently, a large flux of plasma FA

towards the liver exists in these mice. Previously, our group has shown that this mouse model displays severe hepatic steatosis and has a very insulin resistant liver.[20] The peripheral tissues, however, are more sensitive to insulin-mediated stimulation of glucose uptake compared to wild type littermates. The CD36-deficient mice have increased plasma triglyceride levels. The mechanism behind the observed hypertriglyceridemia in *cd36* $^{-/-}$ mice was studied in Chapter 4.

Increased inflammatory cytokine expression levels such as IL-6 and TNFα are associated with insulin resistance.[76,77] Decreased plasma levels of IL-10 have been associated with insulin resistance in humans.[78,79] IL-10 is an immunoregulatory and anti-inflammatory cytokine that can reduce the IL-6 and TNFα production by macrophages.[80-82] To evaluate the possible effects of endogenous IL-10 secretion on insulin sensitivity we compared the effect of high fat feeding on hepatic steatosis and hepatic insulin sensitivity in wild type mouse *versus* the interleukin-10 knock-out mouse (IL-10$^{-/-}$), which completely lacks IL-10 production capacity. In this mouse model we studied the metabolic effects of the absence of IL-10 during high fat feeding (Chapter 5). Our hypothesis was that the IL-10$^{-/-}$ mice would be more insulin resistant.

In Chapters 6 and 7 we used the APOE*3-Leiden transgenic mouse to study the effects of the antiretroviral drug ritonavir (RTV) on the lipid metabolism and the development of atherosclerosis. RTV is a protease inhibitor, which is used in treatment of HIV-infection. The introduction of antiretroviral therapy has led to a significant reduction in the morbidity and mortality that was associated with HIV-infection. Unfortunately, these drugs are associated with severe adverse metabolic effects. Wasting of subcutaneous fat, with or without the accumulation of fat in the dorso-cervical and abdominal region, is frequently observed.[84] Interestingly, like in excess of adipose tissue (obesity), the wasting of subcutaneous fat (lipoatrophy) is also associated with hepatic steatosis.[68,85-87] The metabolic adverse effects also resemble the metabolic disturbances observed in obesity and include hyperlipidemia, hyperinsulinemia and hyperglycemia. RTV use specifically is renowned for the association with severe hypertriglyceridemia.[88] We used the APOE*3-Leiden transgenic mouse to study the underlying mechanisms of RTV-induced hypertriglyceridemia, because this transgenic mouse model is a very well characterized mouse model with a humanized lipoprotein profile.[89-91] Similar to humans, APOE*3-Leiden transgenic mice have a much lower clearance rate of

VLDL-TG than wild type mice. In contrast to wild type mice, these mice are susceptible to diet- and drug-induced hyperlipidemia and to obesity and atherosclerosis. Furthermore, these mice are sensitive to several lipid-lowering drugs such as statins, fibrates and PPARα- and PPARγ-agonists.[92] Consequently, the APOE*3-Leiden transgenic mouse represents a suitable model to investigate the mechanism underlying RTV-induced hypertriglyceridemia. In Chapter 6 we evaluated the cause of hypertriglyceridemia induced by RTV in APOE*3-Leiden transgenic mice. Finally, in Chapter 7, we evaluated the effect of RTV on atherosclerosis in these mice.

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