Aspects involved in the (patho)physiology of the metabolic syndrome

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Chapter 1

General Introduction

In Western society the metabolic syndrome scores high in health risk tables. This syndrome is characterized by a number of metabolic abnormalities like obesity, insulin resistance, dyslipidemia and cardiovascular disease[1-3]. The combination of large amounts of carbohydrates and fats in the Western-type diets and the sedentary life-style are responsible for the fact that in Western society energy intake often exceeds energy expenditure. The excess in lipids and carbohydrates consumed is stored, which in turn can lead to obesity and tissue insulin resistance. The most important dietary nutrients in this respect are cholesterol, triglycerides (TG) and glucose.

Cholesterol is a lipid essential for biosynthesis of cellular membranes, steroid hormones and bile acids. However, high plasma cholesterol levels (hypercholesterolemia) are a risk factor for cardiovascular disease. TG, and their metabolites fatty acids (FA), are lipids that are mainly used for energy. In addition, FA have an important function in regulating gene expression, but in their free form FA are toxic to cells. TG are the form in which FA can be stored in the cell or be transported in the circulation. Especially cardiac and skeletal muscle are greatly dependent on this form of energy. High plasma levels of TG (hypertriglyceridemia) are a risk factor for the metabolic syndrome and, eventually, cardiovascular disease. Glucose is a small carbohydrate, which can be quickly converted into energy. Especially brain and muscle use glucose for energy. Plasma glucose levels are strictly regulated for normal function of the body. Both high and low blood glucose levels can have severe health implications.

These nutrients are all essential in the human body, however their levels need to be kept within certain ranges.

Lipid metabolism

Lipoprotein metabolism

Cholesterol and TG are transported in the circulation in the form of water-soluble spherical particles, called lipoproteins. Lipoproteins have a hydrophobic lipid-rich core, containing mainly TG and esterified cholesterol, surrounded by a polar surface monolayer, which is composed of phospholipids, free cholesterol and several proteins, termed apolipoproteins (apo)[4-6]. Lipoproteins can be divided into five major classes, which differ in origin, density, size and (apolipoprotein) composition[5,7]. As shown in **Table 1** these lipoprotein classes are: chylomicrons (CM), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL)[7].

The various apolipoproteins found in different combinations on lipoproteins have several distinct functions: they either stabilize the lipoprotein particles, serve as ligands for lipoprotein receptors or are cofactors/inhibitors of enzymes involved in lipoprotein metabolism such as lipoprotein lipase (LPL)[7,8].

The metabolism of lipoproteins can be divided in three pathways: the (nutrition-related) exogenous pathway, the endogenous pathway and the reversed cholesterol

transport pathway. In **Figure 1** these pathways are successively presented in a simple form.

Table 1. Human plasma lipoproteins: physical properties and composition

	CM	VLDL	IDL	LDL	HDL
Physical properties					
Source	Intestine	Liver	VLDL	VLDL/IDL	Liver and Intestine
Diameter (nm)	75 - 1200	30 – 80	25 - 35	18 - 25	5 - 12
Density (g/ml)	< 0.96	0.96 - 1.006	1.006 - 1.019	1.019 - 1.063	1.063 - 1.21
Composition					
Total lipid (%)	98 - 99	90 – 94	89	79	45 - 55
Protein (%)	1 - 2	6 – 10	11	21	45 - 55
Lipid Composition (% of total lipid)					
Triglycerides	88	56	29	13	15
Cholesterol esters	3	15	34	48	30
Free cholesterol	1	8	9	10	10
Phospholipids	8	20	26	28	45
Apolipoprotein Content					
Apo	A1,A4,B48, C1,C2,C3,E	B100,C1,C2, C3,E	B100,C1,C2, C3,E	B100	A1,A2,A4,C1, C2,C3,E

Apo apolipoprotein, CM chylomicron, HDL high density lipoprotein, IDL intermediate density lipoprotein, LDL low density lipoprotein, VLDL very low density lipoprotein

Exogenous pathway

Intestinal absorption of dietary lipids is facilitated by intestinal bile acids, lipases and proteases, which are supplied by bile and pancreatic juices released into the small intestinal lumen[9]. TG digestion, by lipases, leads to two free FA and a glycerol-FA. These molecules and cholesterol are absorbed by the epithelial cells of the small intestine (enterocytes). Inside the enterocytes, the FA are re-esterified into TG and are packaged into CM. CM are very large lipoprotein particles containing mostly TG but also cholesterol, phospholipids, and several proteins (apoB48, A1, and A4)[10]. The intestinal epithelial cells secrete the CM into the lymph, which drains into the circulation[11]. Upon entering the bloodstream, the particles acquire apoE, C1, C2 and C3. In the bloodstream the TG are hydrolyzed into free FA and glycerol by endothelium-bound LPL[12,13], allowing the delivery of FA, to the adjacent tissues like muscle (for energy) and adipose tissue (for storage). Upon hydrolysis of the core lipids, the lipoprotein particle becomes smaller and is called CM-remnant. Part of the excess surface material, such as phospholipids, free cholesterol and apolipoproteins, is transferred to HDL particles. The CM-remnant, relatively enriched in cholesterol, is rapidly cleared by the liver via apoE-mediated binding to specific lipoprotein receptors *i.e.*, the LDL receptor (LDLR) and LDLR-related protein (LRP)[12,14].

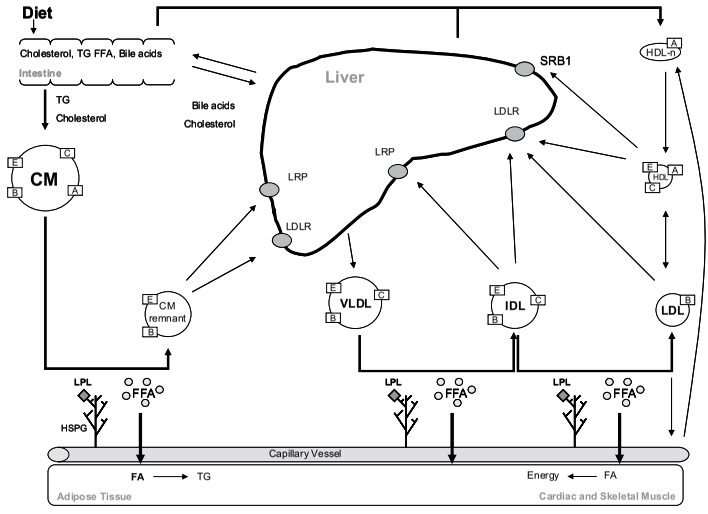

Figure 1. Schematic illustration of the pathways in lipid metabolism: exogenous, endogenous and the reverse cholesterol pathway
CM chylomicron, FA fatty acid, FFA free FA, (n-)HDL (nascent-) high density lipoprotein, HSPG heparin sulphate proteoglycans, IDL intermediate density lipoprotein, LDL low density lipoprotein, LDLR LDL receptor, LPL lipoprotein lipase, LRP LDLR-related protein, SRB1 scavenger receptor B1, TG triglyceride, VLDL very low density lipoprotein

Endogenous Pathway

The liver plays a major role in lipid metabolism. It takes up CM-remnant particles containing mainly cholesterol and it secretes VLDL-particles. VLDL consist of a core of TG and cholesterol, which are newly synthesized by the liver or derived from incoming CM-remnants, IDL, LDL and HDL. In the process of VLDL formation the major structural apolipoprotein of VLDL, apoB100, associates with the core lipids catalyzed by microsomal triacylglycerol transfer protein (MTP). Thereafter, the particle fuses with a lipid droplet to become a mature VLDL particle, which, with help of apoE[15], can be secreted into the blood[16,17]. Upon entering the circulation, the particle is enriched with apoE, and apoC. Like CM, VLDL particles serve as TG transporters to supply the periphery with energy in the form of FA. In a similar way to CM, hydrolysis of VLDL-TG by LPL results in smaller particles called IDL, which can either be taken up by the liver via the LDLR or LRP, or be further hydrolyzed by LPL and hepatic lipase into LDL. LDL is a small lipoprotein particle that has lost most of the apolipoproteins. ApoB100 remains associated with the particle and serves as a ligand for the uptake of LDL via the LDLR present on the liver and peripheral tissues[14,18]. In the human circulation LDL is the most abundant lipoprotein.

Reverse cholesterol pathway

Through the uptake of LDL particles by the vessel wall, cholesterol is present in the subendothelial space. There it is used, or is transported back to the liver, by nascent-HDL (n-HDL) via the so called reverse cholesterol transport pathway. n-HDL is a very small lipoprotein and contains apoA1 as its major apolipoprotein. n-HDL is synthesized by the liver and the small intestine and incorporates redundant surface lipids and apolipoproteins freed during lipolysis of TG-rich lipoproteins[19,20]. Through interaction with the ATP-binding cassette transporter A1 (ABCA1), the cellular cholesterol is taken up in the core of n-HDL in the circulation[21]. By this process, n-HDL is converted into mature (filled) spherical HDL. The cholesterol esters in the mature HDL are taken up by the liver via the scavenger receptor B1 (SR-B1) either directly or transferred from HDL to VLDL and LDL in exchange for TG. The cholesterol esters are then taken up via the classical LDLR- and LRP-mediated pathway.

Cholesterol metabolism

The liver plays a major role in the whole body cholesterol homeostasis. It takes up cholesterol from CM- and VLDL-remnants, from LDL and from HDL. The liver uses cholesterol for the synthesis of VLDL, but the major portion of liver cholesterol is used for the production of bile salts for excretion, together with biliary cholesterol, into the intestine. Approximately 95% of the bile acids are reabsorbed in the terminal ileum and return to the liver, where they are actively taken up. Biliary cholesterol delivers a large amount of the daily intestinal cholesterol and about 50% of the intestinal cholesterol is reabsorbed. This pathway, which is called the enterohepatic circulation, contributes greatly to plasma cholesterol levels[22,23]. Although bile acid and biliary cholesterol excretion is the only

quantitatively significant cholesterol removal pathway, recent work indicates that there might be a bile-independent cholesterol efflux towards the intestine (personal communication Dr. AK Groen, AMC, Amsterdam).

The *de novo* bile acid biosynthesis is initiated by the enzymes 7α-hydroxylase or sterol 27-hydroxylase. After excretion in the intestine, bile acids play an important role in the solubilization of fats, cholesterol and other lipophillic compounds such as drugs and vitamins A, D, E and K, enhancing their uptake by enterocytes.

In humans, approximately 1 gram of cholesterol is needed per day to maintain the enterohepatic circulation. The lipoprotein-derived cholesterol uptake by the liver from in the circulation is not sufficient for this pathway. Per day, the liver synthesizes approximately 700 mg cholesterol in order to maintain the enterohepatic circulation. The major rate-limiting enzyme in cholesterol production is 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Inhibition of this enzyme *in vivo* by administering statins is currently the most commonly used approach for lowering plasma cholesterol.

Triglyceride and fatty acid metabolism

As depicted in **Figure 1**, dietary TG (exogenous TG) and TG synthesized in the liver (endogenous TG) are secreted into the circulation in the form of CM and VLDL, respectively. Plasma TG are lipolyzed and the FA generated are taken up by the adjacent tissues, *e.g.*, muscle for energy and adipose tissue for storage. For the lipolysis of TG the most crucial enzyme is LPL.

Lipoprotein lipase

LPL is an enzyme that belongs to a family of lipases, which also includes hepatic lipase and pancreatic lipase. LPL is synthesized and secreted by almost all tissues in the body, but most abundantly in skeletal and cardiac muscle and adipose tissue. Once secreted, it associates with the heparin sulphate proteoglycans (HSPG) of endothelial cells (**Figure 1**). LPL can not only interact with lipoproteins, it also interacts with lipoprotein receptors, thereby enhancing binding and internalization of lipoproteins.

Active LPL is a homodimer and its activity is influenced by many factors. Of the apolipoproteins residing on the lipoproteins, apoC2 is an essential cofactor for normal LPL function. ApoC1 and apoC3 are natural inhibitors of LPL, of which apoC3 is the most potent[24]. Research is ongoing on novel discovered apolipoproteins like apoA5[25,26], which has a stimulatory effect on LPL activity.

LPL activity is greatly regulated by the nutritional status. In the fed state LPL is highly expressed in adipose tissue under the influence of insulin. On the other hand, during fasting and exercise LPL activity is increased specifically in muscle[27]. Therefore, LPL is a key player in the partitioning of FA[27] and might have a large role in the aetiology of obesity[27-29].

Adipose tissue

Adipose tissue has long been regarded as a mere storage depot, but increasing evidence presents adipose tissue as a highly metabolically active tissue. Adipocytes are nowadays known to produce hormones (like leptin, adiponectin and resistine), cytokines (like TNFα, IL-6 and IL-10), growth factors, complement factors and prostacyclins, collectively called adipokines[30-32]. More molecules are added as research progresses. These adipokines play roles in whole-body insulin sensitivity and metabolic homeostasis. For instance, the hormone leptin regulates, among others, appetite and body weight, and adiponectine is known to be an insulin sensitizer. The cytokines are very important in inflammatory status. Dysregulation of cytokines or other adipokines can lead to great metabolic changes[30-32].

Upon lipolysis of CM- and VLDL-TG in the capillary bed by LPL, FA are taken up by the adipocytes and re-esterified in the form of TG. This process requires glucose for the formation of glycerol to which the FA are esterified (**Figure 2**). Vast amounts of TG are stored for later use, e.g., during fasting. Upon fasting, FA are liberated from the adipose tissue by the action of hormone sensitive lipase (HSL)[33] and the recently discovered adipose tissue TG lipase (ATGL)[33-35]. The free FA are released into the circulation, where they bind to albumin for transport.

Cellular fatty acid handling

The major portion of albumin-bound FA is transported to the liver. In the liver, these FA are re-esterified in TG and are used for production of VLDL. In the fasted state, muscle cells take up FA generated from the lipolysis of liver-derived VLDL (**Figure 1** and **2**). This FA uptake is mediated by several FA transporters as well as passive diffusion. The three main FA transporters are plasma membrane FA binding protein (FABPpm), FA translocase (FAT/CD36) and FA transport protein (FATP). Once inside the cell, cytosolic FABP (FABPc) binds the FA for transport through the watery environment of the cytosol toward the mitochondria. The FA must first be activated in the cytoplasm before they can be oxidized in the mitochondria for energy[36-38]. In the presence of adenosine triphosphate (ATP) and coenzyme A (CoA), this activation is catalyzed by fatty acyl-CoA synthase (FACS) resulting in an acyl-CoA (**Figure 3**)[39,40]. The acyl-CoA esters are directed to peroxisomes and mitochondria for β-oxidation or can serve as substrates for TG, phospholipids, and cholesterol ester synthesis. Under fasting conditions however, they are channeled primarily toward mitochondria for β-oxidation. Long-chain FA will not penetrate the inner membrane of mitochondria to be oxidized, unless they are converted to an acyl-carnitine intermediate. Therefore, carnitine palmitoyltransferase (CPT) I, localized in the outer mitochondrial membrane, couples the long-chain acyl-CoA to carnitine to form acyl-carnitine. This carnitine ester can be transported across the mitochondrial inner membrane by the carnitine/acylcarnitine translocase (CT). Inside the mitochondrial lumen, CPTII uncouples the acyl-CoA, which serves as a substrate for the β-oxidation spiral (**Figure 3**).

β-oxidation of the acyl-CoA involves successive cleavages releasing acetyl-CoA, by enzymes specific for the chain-length of the FA. Eventually, this reaction generates a large quantity of ATP, the energy-rich compound used for cellular reactions.

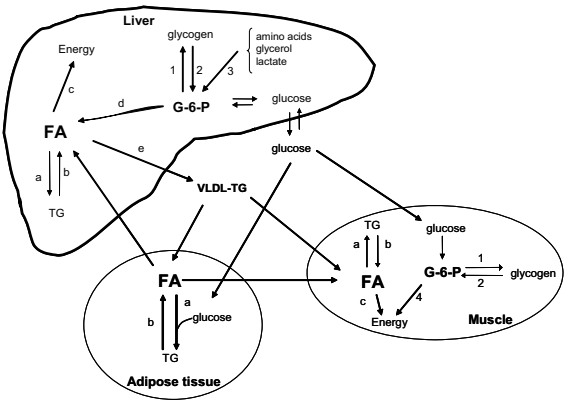

Figure 2. Schematic illustration of FA and glucose metabolism
Glucose metabolism: 1 glycogenesis, 2 glycogenolysis, 3 gluconeogenesis, 4 oxidation.
Lipid metabolism: a lipogenesis, b lipolysis, c oxidation, d *de novo* FA synthesis, e VLDL production.
FA fatty acid, TG triglycerides, G-6-P glucose-6-phosphate, VLDL very low density lipoprotein

Ketogenesis

During high rates of FA oxidation large amounts of acetyl-CoA are generated primarily in the liver. When the acetyl-CoA supply exceeds the energy needs of the liver itself, they are used for keton body (acetoacetate, β-hydroxybutyrate, and acetone) synthesis. Keton bodies can be used as fuel by tissues other than liver. In early stages of starvation, when the last remnants of fat are oxidized, heart and skeletal muscle will consume primarily keton bodies to preserve glucose for use by the brain, eventually the latter will use keton bodies as well.

In diabetes mellitus patients, high levels of FA oxidation may lead to deadly diabetic ketoacidosis.

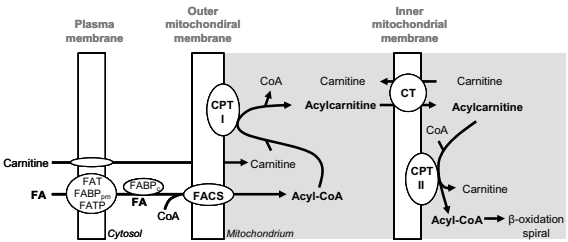

Figure 3. Schematic illustration of FA transport and handling in cells
CoA coenzyme A, CPT carnitine palmitoyltransferase, CT carnitine/acylcarnitine translocase, FA fatty acid, FABP FA binding protein, $FABP_c$ cytosolic FABP, $FABP_{pm}$ plasma membrane FABP, FACS fatty acyl-CoA synthase, FAT FA translocase, FATP FA transport protein

Glucose metabolism

Next to lipids, glucose is an important constituent of our diet and is a major source of energy. Metabolically active tissues like adipose tissue, liver and muscle need at least a small amount of glucose to maintain their basal functions. For some organs, like the brain, glucose is essential for proper functionality. Next to uptake via the diet, glucose can also be synthesized by the liver (and to lesser extent by the kidneys). Besides being oxidized for energy, glucose can also be stored in the form of glycogen. Blood glucose levels need to be strictly regulated to maintain proper physiology. Too low levels (hypoglycemia) and too high levels (hyperglycemia) can give rise to many complications.

Regulation of the blood glucose

After a meal, glucose and other carbohydrates are absorbed in the intestine and secreted into the blood via the portal vein. Via the glucose transporter GLUT4 the adipose and muscle tissues are able to take up glucose under the influence of insulin[41,42]. In the cell, glucose is used as fuel or is stored in the form of glycogen (in muscle) for later use. It can also be used for *de novo* lipogenesis.

Hepatic glucose metabolism includes uptake of glucose from the portal circulation via insulin-independent transporters like GLUT2 and *de novo* synthesis. The liver is capable of storing considerable amounts of glucose in the form of glycogen. During fasting, plasma glucose levels are maintained by the liver. First by glycogenolysis, and later by gluconeogenesis[43] (**Figure 2**). Under normal conditions, plasma glucose is kept within a strict range by the use of several hormones and nervous signals. This strict regulation is

important since too low levels of glucose prohibit normal function of brain (and some other tissues) resulting in loss of consciousness and in severe cases even in coma. On the other hand raised blood glucose levels (>7 mmol/l in fasted state) induce thirst, polyuria and in the long run macro- and microvasculair damage *e.g.*, atherosclerosis, nefro-, neuro- and retinopathy[44-46].

The hormone insulin is the most important player in the regulation of blood glucose levels and is the only glucose-lowering hormone. Other pancreatic hormones, like glucagon and somatostatin, are able to increase plasma glucose levels. Insulin is synthesized in the β-cells of the pancreas and is released in the blood in response to increasing plasma glucose levels after a meal. Insulin interacts with insulin receptors on the muscle and adipose tissue and stimulates these tissues to take up glucose by increasing the number of GLUT4 transporters. As a result, blood glucose levels and insulin secretion will decrease. Next to increasing body glucose uptake, insulin stimulates liver glycogen synthesis and decreases the hepatic glucose production and the VLDL-secretion. Furthermore, in the fed state, in adipose tissue HSL is inhibited by increased insulin levels, which leads to decreased TG lipolysis and FA secretion, as well as, increased esterification of FA in adipose tissue. The latter process needs glucose for glycerol production. The uptake of glucose by the adipose tissue is stimulated by the increased insulin levels. In this way after a meal, the insulin level ensures that: 1) dietary glucose is taken up by muscle (for energy) and adipose tissue (for FA esterification), 2) hepatic output of glucose is inhibited, concomitant with an increased conversion of hepatic glucose into glycogen and 3) lipolysis of CM- and VLDL-TG is enhanced at the adipose tissue, whereas this is decreased in muscle.

Thus, although the major physiological function of insulin is the maintenance of plasma glucose homeostasis, insulin also plays an important role in lipid metabolism[43,47]. In summary: Insulin ensures glucose and FA uptake from the diet whereafter these two fuels are used for energy and storage, respectively.

Hepatic glucose metabolism

As mentioned above glucose can be synthesized in the body by the liver. The rate of hepatic glucose production is an important determinant of blood glucose levels. In the fed state glucose enters the hepatocyte and is readily converted into glucose-6-phosphate by glucokinase (**Figure 2**). Glucose-6-phosphate is an important regulator of hepatic glucose metabolism. It can be oxidized via glycolysis, leading to formation of pyruvate, acetyl-CoA and finally energy. Glucose-6-phosphate can also be converted and stored in the form of glycogen.

During the fasting state, when energy supplies from dietary sources become limited, glucose can rapidly be mobilized from the hepatic glycogen stores (glycogenolysis). During glycogenolysis, glycogen is converted into glucose-6-phosphate and subsequently into glucose, which is released into the circulation. When hepatic glycogen reserves become

depleted, after 12 hours of fasting in the human situation, the process of glucose synthesis (gluconeogenesis) becomes accelerated.

Major precursors for glucose synthesis are lactate (the end product of anaerobe dissimilation of glucose in red blood cells and muscle) and glycerol (from lipolysis of TG in adipose tissue). In the fed state, when insulin levels are high and glucagon levels are low, glucose-6-phosphate is prone to glycolysis and glycogen synthesis. Activity of key enzymes involved in these processes, respectively phosphofructokinase-1 and glycogen synthase, is increased. This results in diminished glucose production.

During fasting, when insulin levels are low and glucagon levels are high, the processes that capture glucose-6-phosphate within the liver are low in activity. Under these circumstances, the activity of key enzymes involved in the process of glycogenolysis (glycogen phosphorylase) and glucogenesis (phosphoenol pyruvate carboxykinase - PEPCK) is increased. This results in enhanced glucose release by the liver.

Transcription factors

Much attention in research is focused on transcription factors, since they are known to be able to regulate important genes in several pathways involved in lipid and glucose homeostasis. These factors are differentially expressed in the cells of tissues. In the nucleus the transcription factors (or complexes of transcriptions factors heterodimerized with retinoid X receptor (RXR)[48]) bind to a specific responsive element in the promoter region of the target gene. Upon activation of the transcription factor or RXR the expression of the target gene is modulated.

Peroxisome proliferator-activated receptor

Many studies have been performed on the transcription factors named peroxisome proliferator-activated receptors (PPARs). Three PPAR isotypes have been identified namely: PPARα, PPARβ and PPARγ[49,50]. PPARs heterodimerize with RXR. The natural ligands for PPARs seem to be long-chain unsaturated FA such as linoleic acid, phytanic acid, conjugated linoleic acid and eicosanoids. Since PPARα and PPARγ have been shown to regulate genes involved in the FA oxidation pathways, focus is maintained on these two isotypes of the PPARs.

PPARα is highly expressed in adipose tissue and liver, and to a lower extent in kidney, heart and skeletal muscle. Next to FA, fasting conditions and fibrates are able to activate PPARα. The target genes of PPARα are a relatively homogenous group of genes involved in lipid catabolism, such as FAT and FATP, liver-FABP, FACS, CPTII, LPL and apoC3.

PPARγ is mainly expressed in adipose tissue, and to a lesser extent in liver, skeletal muscle, colon, the immune system and the retina[49]. PPARγ controls cellular differentiation, TG synthesis and lipid storage, and modulates the actions of insulin

(*i.e.,* by upregulating GLUT4)[50]. Insulin sensitivity can be improved using glitazones (anti-diabetic drugs) that are high affinity ligands for PPARγ[51].

Liver X Receptor

The Liver X receptors (LXRs), LXRα and LXRβ, are also transcription factors involved in the regulation of lipid metabolism. LXRα is highly abundant in liver and other tissues involved in lipid metabolism, whereas LXRβ is ubiquitously expressed. Both LXRα and LXRβ form obligate heterodimers with RXR and can be activated by lipids, *i.e.*, oxysterols, which are intermediates in cholesterol metabolism in the liver, adrenal glands and brain. Studies in mice have shown that ABC transporters and CYP7A1 (the 7α-hydroxylase gene) are target genes of LXR. Furthermore, an overlap between LXR and PPAR signaling pathways has been suggested, as well as activation of SREBP1c (see below) by LXR, indicating that LXRs are important and very complex factors in lipid homeostasis[52].

Farnesoid X Receptor

The farnesoid X receptors (FXRs) control bile acid as well as lipid metabolism and recent observations indicate even a role in carbohydrate metabolism[53]. They modulate the expression of a wide variety of target genes by binding either as a monomer or as a heterodimer with RXR. FXR is highly expressed in liver, intestine, kidney and the adrenal glands with lower levels in fat and heart. Bile acids have been identified as natural ligands for FXRα[54].

Sterol Regulatory Element Binding Protein

SREBPs (sterol regulatory element binding proteins) are transcription factors that can directly activate the expression of genes involved in synthesis and uptake of cholesterol, FA, TG and phospholipids. Two isotypes have been identified, SREBP1 (among which SREBP1a and SREBP1c) and SREBP2. SREBP1c preferentially regulates FA-biosynthetic pathways and SREBP2 favors cholesterol synthesis. SREBP1a is able to activate both pathways. Important target genes of SREBPs are fatty acid synthase (FAS), LDLR, LPL and HMGCoA synthetase and reductase[55,56].

Disorders in lipid and glucose metabolism

Glucose and lipid metabolism are interconnected complex processes designed to maintain energy homeostasis. Many different factors can lead to dysregulation of these processes with various serious consequences. Below, a number of metabolic abnormalities are briefly summarized: obesity, insulin resistance, type 2 diabetes, dyslipidemia and atherosclerosis. Together, these interconnected metabolic dysregulations represent the various aspects of the metabolic syndrome. During the last decades, the incidence of the metabolic syndrome has taken epidemic proportions. Whereas obesity, type 2 diabetes and dyslipidemia were long thought to be separate diseases, now they have been classified under the metabolic syndrome, or syndrome X. Debates are held on what exact definition is to be used. The world health organization (WHO) definition is: occurrence of diabetes or impaired fasting

glucose or impaired glucose tolerance or insulin resistance, plus two or more of the following: obesity (body mass index > 30), dyslipidemia (hypercholesterolemia, hypertriglyceridemia, low HDL levels), hypertension, microalbuminuria[1]. Since this syndrome is affecting more and more people, much research is being performed on revealing the connections between the different symptoms. Patients presenting one of the symptoms need to be checked for other features of the metabolic syndrome, since it is very unlikely they suffer from only a single disorder[1-3].

Obesity

Obesity, nowadays a common phenotype, is characterized by increased amounts of adipose tissue. The body mass index (BMI) is the most common measure to determine if a person is considered obese. Generally a BMI (calculated by body mass in kg/square of the height in m) over 30 is indicative of health-impairing obesity. Unfortunately, BMI is a crude measurement and other techniques are available to determine the severity of the obesity.

Next to psychosocial problems, obesity leads to painful joints, elevated plasma free FA, insulin resistance, hypertention, heart disease and many other metabolic dysregulations and diseases.

The distribution of the excess fat needs to be considered since body fat pads are roughly divided in visceral adipose tissue (abdominal or central fat), and subcutaneous adipose tissue (peripheral fat), which are metabolically different tissues. Visceral fat is more sensitive to the lipolytic effect of catecholamines and less sensitive to the antilipolytic and TG-storing effect of insulin compared to subcutaneous fat[57,58]. This difference, and the fact that visceral fat directly drains into the portal vein, leads to a relatively high exposure of the liver to visceral adipose tissue-derived free FA (see dyslipidemia) and/or adipokines[59]. Increased levels of leptin, resistine and cytokines, and decreased levels of adiponectin have been described for obese patients, generating metabolic dysregulation and an increased inflammatory state[30-32].

Although obesity can be the result of both genetic and environmental factors, the Western world diet and sedentary life-style are the predominant causes of obesity. Life-style changes and medication have proven to be effective in decreasing obesity in patients[57].

Insulin resistance

Obesity is often accompanied with TG accumulation in other tissues. TG accumulation in turn, often leads to insulin resistance in the respective tissues. Insulin resistance is characterized by unresponsive of the tissue to the actions of insulin. Therefore, more insulin is needed to maintain proper glucose homeostasis in the cells and plasma.

Several conditions are known to induce insulin resistance in tissues, especially TG storage in tissues other than adipose tissue. The liver is able to store rather large amounts of TG for later use. However, it has been recognized that excessive liver lipid accumulation (hepatic steatosis) is linked to hepatic insulin resistance[60-63], leading to increased hepatic

glucose production and VLDL production. Impaired hepatic insulin signaling seems to underlie this steatosis-induced insulin resistance in the liver[61].

In muscle, excess TG storage is also known to induce insulin resistance, although underlying mechanisms are still under debate[60,63-65].

Type 2 diabetes

The disease type 2 diabetes was known as a disease of the aging, since the pancreatic β-cells lose their ability to produce insulin over time. In the last decades patient numbers have explosively increased in all age-groups. This complex disease is a feature of the metabolic syndrome and is often associated with obesity. In type 2 diabetes, the pancreas usually is able to secrete insulin, however there is an imbalance between the capacity for insulin production and the responsiveness of tissues to insulin. When tissues are insulin resistant, the pancreatic production is increased in order to counteract this unresponsiveness, leading to hyperinsulinemia. In time, the pancreatic β-cells are unable to cope with the increased demand, which leads to their destruction. During the different stages of type 2 diabetes different treatment strategies are available, ranging from life-style adjustments, to oral medication (like thiazolidinediones or metformin). Insulin supplementation, however is usually inevitable in time[66].

Growing evidence over recent years supports a potential role for cytokine-associated, subacute inflammation in the pathogenesis of insulin resistance and type 2 diabetes. For example, the cytokine NFκB induces the expression of hepatocyte-specific target genes involved in the pathogenesis of type 2 diabetes (insulin resistance, increased VLDL-TG levels, and hepatic steatosis)[67-69].

Dyslipidemia

Disturbances in plasma lipid levels are known to lead to metabolic problems. Increased free FA levels are associated with obesity, hepatic steatosis and insulin resistance. In addition, increased plasma FA levels are often associated with hypertriglyceridemia[70]. Several genetic forms of dyslipidemia have been described[71]. However, increased plasma lipid levels may be the result of increased dietary intake, altered handling, or increased endogenous production. In hypertriglyceridemia the increased plasma TG levels are usually confined to the VLDL lipoprotein fraction caused by either increased VLDL-TG production or decreased VLDL-TG lipolysis and clearance.

The VLDL production rate is dependent on the hepatic lipid pool. This pool is the net result of hepatic FA uptake and lipogenesis on one hand, and FA oxidation and ketogenesis on the other hand. Also, VLDL production is suppressed by insulin, thus in insulin resistant states the regulation (suppression) of hepatic VLDL production by insulin is diminished. Several studies found a correlation between hepatic VLDL production and insulin resistance and type 2 diabetes[72,73].

In obese people, portal FA flux toward the liver from visceral adipose tissue is increased. In combination with hepatic insulin resistance this leads to increased lipogenesis and decreased FA oxidation giving rise to increased amounts of hepatic TG.

Additionally, it has been postulated that there is decreased LPL activity in insulin resistant tissue. Therefore, lipoproteins are less efficiently lipolyzed, resulting in larger lipoprotein-remnants. Due to the larger particle size, the uptake of these particles is decreased, also adding to the hypertriglyceridemia.

Atherosclerosis

Hyperlipidemia is the major cause of atherosclerosis and, eventually, cardiovascular disease. Increased levels of cholesterol-rich particles (CM- and VLDL-remnants and LDL) in the plasma results in increased penetration of these lipoproteins into the vessel wall. Modification of these lipoproteins in the subendothelial space or intima leads to excessive accumulation of cholesterol in the residing macrophages and conversion of these cells into foam cells. The formation of foam cells in the intima is commonly considered as the very initial step in atherosclerotic plaque formation. Foam cells in the intima produce inflammatory cytokines and chemotatic molecules. Inflammatory cells are recruited, leading to further growth of the lesion/plaque in the arterial wall. The bloodflow in the artery will become impaired. Moreover, rupture of the lesion may cause thrombus formation, leading to cardiovascular events, such as myocardial infarction and stroke[74,75].

Outline of the Thesis

The studies described in this thesis are aimed at unraveling the metabolic relationship between various aspects of the metabolic syndrome, like obesity, insulin resistance, hepatic steatosis and dyslipidemia.

In **chapter 2** our aim was to study whether the absence of apoC3, a strong inhibitor of LPL, accelerates the development of obesity and, consequently, insulin resistance. We hypothesized that the redistribution of plasma TG in *apoc3*$^{-/-}$ mice on a high-fat diet leads to weight gain. In these mice and wild type littermates we followed the development of features of the metabolic syndrome, *e.g.,* levels of plasma lipid, glucose and insulin, obesity and body composition, and tissue-specific insulin resistance.

Hepatic VLDL and glucose production is enhanced in type 2 diabetes and is associated with hepatic steatosis. In **chapter 3** we used methyl palmoxirate to acutely inhibit hepatic FA oxidation, and investigated whether changes in hepatic β-oxidation influence VLDL production/secretion, and whether this would affect hepatic steatosis and glucose production *in vivo*.

Dietary FA have profound impact on the occurrence of hyperlipidemia and/or hepatic steatosis, but mechanisms are not fully understood. In **chapter 4** we studied the effects of a saturated-fat diet, supplemented with fish oil, *trans*10,*cis*12-conjugated linoleic acid (CLA), or elaidic acid, on lipid and glucose metabolism in APOE*3Leiden mice. In

addition, by using the proteomic approach we measured numerous liver proteins of these mice to increase insight in the biochemical pathways underlying the metabolic relationship between dietary FA and hepatic lipid and glucose metabolism.

Sphingolipids are lipids found as membrane constituents in plants, yeasts and animals and are present in our daily diet. The sphingolipid sphingomyelin has been shown to decrease plasma cholesterol in rats. In **chapter 5** we questioned whether various sphingolipids supplemented to the Western-type diet decrease plasma cholesterol and/or TG in hyperlipidemic APOE3*Leiden mice. More specifically, we wondered if sphingolipids added to the diet could be used to treat the dyslipidemia and the related abnormal hepatic lipid homeostasis, characteristic of the metabolic syndrome.

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